Differentiation of Smooth Muscle Cells in Human Atherectomy Specimens: Implications for Restenosis and Primary Arteriosclerosis

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Abstract

Pathogenic mechanisms responsible for restenosis and arteriosclerosis in man are still incompletely understood. Phenotype conversion of smooth muscle cells (SMCs) has been suggested as essential prerequisite for subsequent migratory and proliferative events leading to neointima formation. To quantify features of SMC differentiation and to relate them to specific lesion types, we analyzed atherectomy specimens from 14 restenotic and 27 primary coronary and peripheral lesions, using transmission electron microscopy. We found that restenotic hypercellular plaques contained closely packed SMCs rich of synthetic organelles, only partly surrounded by desintegrated, fragmented extracellular matrix components. As the central finding, SMC volume fraction of synthetic organelles (V_s) was 2.2 fold greater in restenotic than in primary plaque tissue, indicating a decrease in SMC differentiation. Enhanced V_s values were equally seen for restenotic coronary and peripheral lesions, showed no decrease with postangioplasty time (2.2 to 30 months), and closely resembled V_s values found for arterial SMCs in culture. The present morphometric study of human atherectomy specimens demonstrates for the *in vivo* and *in vitro* situation that a decrease in SMC differentiation is an early, long-lasting biologic feature in response to injured SMC microecology.

Key words: arteriosclerosis, human smooth muscle cell differentiation, restenosis.

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Percutaneous balloon angioplasty and novel, second generation techniques offer a less intrusive, distinct treatment of target arteriosclerotic lesions. However, irrespective of their therapeutic modality, all these approaches are still burdened by the problem of 30-50% subsequent restenosis [1, 14, 30, 33, 35, 52, 55, 57, 64, 65]. Therefore, to develop more specific and effective anti-restenotic strategies, an improved understanding of the basic pathobiologic processes involved in human restenosis and preexistent arteriosclerosis is needed.

Analysis of human autopsy material and plaque specimens retrieved by directional atherectomy has turned out to be an invaluable means to identify structural and functional elements implicated in restenosis, especially for the comparative examination of restenosed intimal tissue to that of primary lesions [3-6, 9, 19, 22, 32, 34, 37, 45, 49]. By histology and immunohistochemistry, smooth muscle cells (SMCs) were proven to be the predominant cell type in human target lesions of primary and restenotic origin, with an excess of SMCs in the latter [3, 22, 32, 45, 50]. *In situ* hybridization and immunohistochemistry studies, especially those of recurrent lesions, have detected transcripts of PDGF [10, 67, 68], PDGF-R [67], EGF-R [8], TGF-β [44], and proteins of aFGF, bFGF [21] and IGF-1 [26] in human plaque tissue, which indicates a predominantly SMC-localized expression of these specific growth factor ligands and receptors. To elucidate the mechanisms leading to restenotic SMC hypercellularity, assays using SMCs cultured from human atherectomy specimens quantitated SMC proliferative and migratory activity [6, 9, 19, 37, 49]. Comparing SMCs originating from hypercellular restenotic tissue to those from primary lesions revealed a significant increase in mitogenic and migratory potential attributable to restenotic origin [6, 9, 19, 37, 49]. Likewise, several animal models and traumatization modes mimicking different endoluminal interventions were used extensively to study key features and mechanisms of postangioplasty restenosis [2, 43, 56]. These animal and *in vitro* data have suggested modulated SMC differentiation to be a reasonable candidate for the stimulatory capacity of vascular SMCs [24, 38, 58, 63].

Therefore, this study with human coronary and peripheral plaque specimens was performed (i) to systematically and quantitatively analyze subcellular features of rest-

enotic lesions, notably the differentiation pattern of SMCs, and (ii) to compare these data to those found in primary lesions. Increased knowledge of the regulation of SMC (de)differentiation and of the pericellular microecology may allow insight into specific cellular mechanisms and functions. This should be helpful to develop promising and novel therapeutic concepts which target specific forms of human arteriosclerosis, especially of its most aggressive form, restenosis.

Methods

Patient population

A population of 41 patients (27 men and 14 women; median age, 63 years, range 39 to 82 years) were percutaneously treated by directional atherectomy [30, 65]. Tissue specimens were obtained from 12 coronary and 29 peripheral arteries, including 14 restenotic and 29 primary lesions (angiographic stenosis degree > 80%). Plaque material was removed from nine left anterior descending, two right coronary, one circumflex coronary, 26 superficial femoral, one iliac, and two popliteal arteries. Samples of three patients were studied from a primary as well as a subsequent restenotic lesion; one lesion exhibited repeated restenoses and therefore was treated twice with atherectomy. Restenosis was defined according to clinical and angiographic criteria reported previously [40, 57]. Seven restenotic lesions were excised 2.2 to 30 months after initial balloon dilatation, seven lesions 2.4 to 8.2 months after initial atherectomy. The plaque material studied was divided into four groups: Plaque specimens excised from primary and restenotic coronary lesions were designated as groups A and B, respectively, whereas those from primary and restenotic peripheral lesions were designated as groups C and D, respectively. The present study was approved by the ethics committee of our medical faculty, and informed consent for the analysis of removed plaque tissue was obtained from all patients beforehand.

Fixation and transmission electron microscopy

Immediately after percutaneous atherectomy, the retrieved plaque specimens were fixed in 3.5% glutaraldehyde (Merck Inc., Darmstadt, FRG) phosphate buffer (pH 7.4). After 2 hours, the tissue was placed in 1% glutaraldehyde. Additional fixation was performed for 2 hours in 1% OsO_4 (Merck Inc.) in phosphate buffer, and then again thoroughly rinsed in buffer. Tissue samples for transmission electron microscopy were twice dehydrated through graded concentrations of alcohol, and through propylene oxide (Merck Inc.), each for 20 min. After additional incubation in a mixture of propylene oxide and araldit (1:1; Merck Inc.), the blocks were embedded in a mixture of araldit, DDSA and accelerator 964 (Merck Inc.). Polymerization was performed for 48 hours at 60C. Tissue sections (1.0 µm) were cut from the araldit blocks and stained with standard hematoxylin (Merck Inc.) for light microscopy. Ultrathin sections were cut with a diamond knife on an ultramicrotome (Leica Inc., Bensheim, FRG), contrasted by uranyl acetate dihydrate and 1% lead nitrate after Reynolds (Merck Inc.), and then viewed at 80 kV in a Philips CM 10 electron microscope.

For transmission electron microscopy of cultured smooth muscle cells the protocol was modified. Time of fixation with 1% OsO_4 was shortened to 45-60 min. After dehydration through graded alcohol concentrations, the propylene oxide step was omitted and the propylene oxide/araldit mixture was replaced by an alcohol/araldit mixture.

Quantitative analysis of SMC differentiation

Transmission electron microscopic photographs depicting intimal plaque regions were used to evaluate SMC phenotype. The primary magnification was 3600x. Images of randomly selected intimal regions were then photographically enlarged an additional 2.3 times to produce 17 x 21 cm micrographs. Six micrographs were taken for each of a total of 41 lesions. More than 600 plaque cells were classified as either SMC or non-SMC (e.g. macrophage, lymphocyte or endothelial cell). Morphological criteria used for ultrastructural recognition of SMCs were the presence of basal lamina, plasmalemmal vesicles, myofilament bundles, dense bodies [42] and the absence of numerous lysosomal inclusions. Only those cell profiles of sufficient size and quality to allow a distinct evaluation as SMC or non-SMC by these criteria (over 90% of all the detected, cellular elements) were analyzed.

Quantitative assessment of SMC differentiation was performed by morphometric analysis of the SMC volume fraction of synthetic organelles, using a modification of a method previously described [42]. Briefly, synthetic organelles of SMCs were taken to include rough and smooth endoplasmic reticulum, Golgi apparatus, plasmalemmal vesicles, free ribosomes, mitochondria, and glycogen particles. Organelle as well as cytoplasmic volume, excluding lipid droplets, was encircled on each photomicrograph by digital planimetry (Digital-Polar-Planimeter 330E, Haff Inc., Pfronten, FRG). After morphometric quantification of both volumes, the ratio of synthetic organelle volume fraction (V_s) as percentage of SMC cytoplasm was calculated. V_s was measured for each photograph rather than for individual SMCs to avoid sampling bias, since this allowed the analysis of tangential cell profiles sectioned at a distance from the nucleus as well as the ability to avoid overrepresenting the cellular organelles in regions close to the perinuclear region of SMCs. Moreover, to consider intraplaque variability of SMC density and phenotype expression, V_s was calculated as a mean ± SD of six distinct evaluations per experimental sample.

Immunohistochemistry

Monoclonal antibodies specific for smooth muscle cell α-actin (clone asm-1) were obtained from Boehringer Inc. (Mannheim, FRG); the APAAP kit was purchased from Dako Inc. (Hamburg, FRG). Positive immunohistochemical reaction was marked by distinct, red to lilac signals. Nuclear counterstaining was performed by standard hematoyxlin staining. Histologic sections were photographed by

a Nikon Optiphot-2 photomicroscope (Nikon Inc., Düsseldorf, FRG) using Kodak Ektachrome 100 ISO color films. Further methodological details were previously described [39, 62].

Assessment of cell density in histologic plaque sections

Hematoxylin stained, histological sections allowed detection of cellular nuclei from intimal regions of 41 different lesions; adjacent medial parts were not analyzed. Morphometric assessment of cell density was performed by counting stained cell nuclei on a high resolution video monitor (Endovision 534; Storz Inc., Tuttlingen, FRG). The microscopic image (Optiphot-2) was relayed *via* miniaturized video camera (Endovision 534) to a downstream monitor (final magnification, x500). Ten randomly selected intimal areas, each encompassing 0.4 mm^2, were assessed per tissue sample, and the corresponding number of cells was counted. Morphometric examination of intimal cell density was performed by two independent investigators in a double blind fashion.

Cell culture technique

Culture of plaque SMCs from atherectomy specimens was achieved by use of the explant technique with subsequent subculture or by primary, enzymatic disaggregation with a collagenase/elastase mixture, 1.8 mg/ml collagenase [Worthington CLS III (229 U/mg; Biochrom Inc., Berlin, FRG)], 0.2 mg/ml pancreatic elastase (Boehringer Inc.), 1 mg/ml trypsin inhibitor from soybean (Serva Inc., Heidelberg, FRG), dissolved in HEPES-buffered Ham F12 culture medium (Biochrom), pH 7.2. Cells were cultured in a mixture of Waymouth's MB 752/1 (Gibco BRL Inc., Eggenstein, FRG) and Ham F12 media (1:1, vol:vol), buffered with 10 mM HEPES, and supplemented with 15% fetal bovine serum (FBS; Gibco Inc.) and 0.1% tobramycin. Cells were grown on glass coverslips (primary cultures to second subcultures) and maintained at 37C and 5% CO_2. Further methodological details were previously reported [4, 12].

Statistical Analysis

For data analysis the SPSS/PC+ software package was used. Group differences in SMC volume fraction of synthetic organelles V_s were evaluated by the Mann-Whitney *U* test. All probability values were two-tailed and corrected for ties. Values of $p < 0.05$ were considered as significant. Group data are presented as means ± SD.

Results

Morphologic profiles found in human atherectomy specimens - Restenotic versus primary lesions

In this study of plaque tissue from 41 coronary and peripheral arteries, the vast majority of intimal cells were identified as smooth muscle cells, as to the aforementioned ultrastructural criteria or to positive immunohistochemical staining. Representative examples are illustrated in Figures 1-4. Macrophages were only infrequently seen

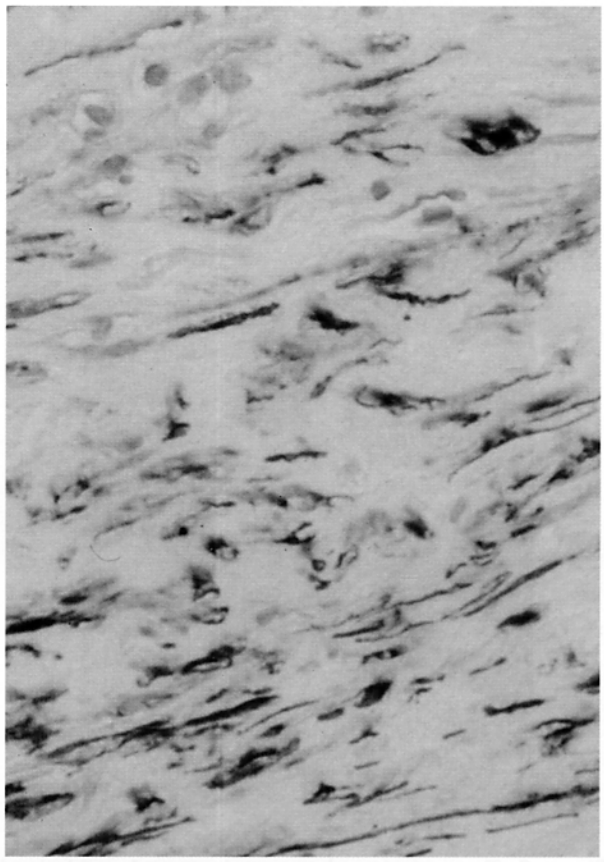

Figure 1. Photomicrograph of the intima histology of a restenotic peripheral lesion exhibiting a typically hypercellular pattern. Positive reaction with antibodies specific for smooth muscle cell α-actin (red signals) identifies the vast majority of intimal cells as smooth muscle cells. Hematoxylin; x250.

(less than 10% of total cell number). These could be identified easily by their lysosome-rich cytoplasm. Likewise, endothelial cells were only rarely detected (two of 41 plaques) and these were marked by distorted integrity.

Smooth muscle cells (SMCs) represented the predominant cell type in high-grade arteriosclerotic lesions for both primary and restenotic plaques. Coronary and peripheral SMCs revealed a broad range of different phenotypes. SMCs with filament-rich cytoplasm and only small organelle volume (contractile to intermediate phenotype SMCs) were infrequently detected in the intima. When present, they were typically found within primary plaques exhibiting sparse cell density and expanded extracellular matrix network (Figure 2a). In the majority of chronic lesions, as a sign of decreased differentiation, SMCs contained organelles such as ribosome-containing membranes of endoplasmic reticulum (RER), Golgi apparatus and mitochondria, as well as microfilament bundles in their periphery (intermediate phenotype SMCs). Figure 2b shows a representative ultrastructural example of these

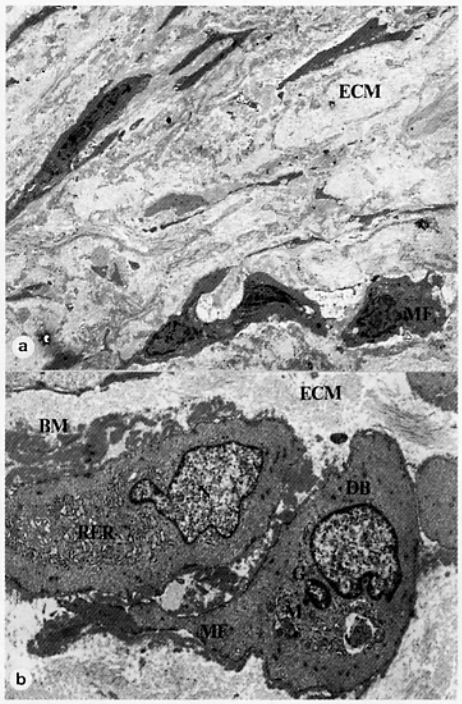

Figure 2. Transmission electron micrographs of intimal portions of (a) coronary and (b) peripheral primary lesions, demonstrating the variable appearance of SMCs.- (a) Extended fields of extracellular matrix (ECM) surround single, slender SMCs with myofilaments (MF) and few organelles (classified as contractile to intermediate phenotype SMCs); x3,800.- (b) Cross-section of intermediate phenotype SMCs filled with numerous myofilaments (MF) and some dense bodies (DB) located in the cellular periphery. In addition, note the presence of cytoplasmic organelles [e.g. mitochondria (M), Golgi apparatus (G) and rough endoplasmic reticulum (RER) membranes] in the perinuclear region.
A thick basement membrane (BM) is deposited in the territory between the SMC surface and the ECM. N = Nucleus; x11,000.

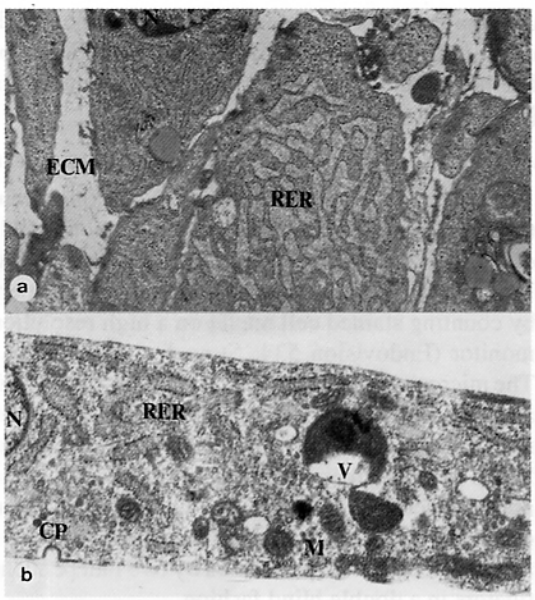

Figure 3. Transmission electron micrographs of (a) intimal portions of a restenotic lesion and (b) human arterial SMCs in culture, indicating increased metabolic activity of SMCs. - (a) Hypercellular femoral plaque tissue, with a remarkably high volume fraction of synthetic organelles predominantly comprising RER membranes which extent to peripheral portions of each cell (synthetic phenotype SMCs). Intercellular space was characterized by modest, residual components of a severely desintegrated ECM. N = nucleus; x16,800.- (b) Ultrastructural detail of a cultivated SMC after enzymatic removal of ECM network. Presence of numerous ribosome-occupied membranes of the endoplasmic reticulum (RER), mitochondria (M), clathrin-coated pits (CP) and vesicles (V) give evidence of an ongoing metabolic activity of the SMC in culture. N = nucleus; x34,500.

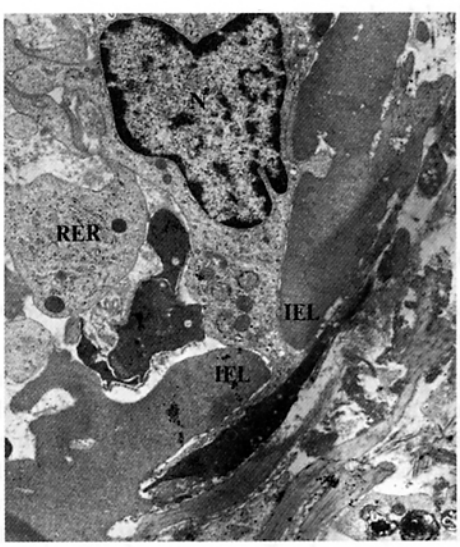

Figure 4. Transmission electron micrograph of deep intimal portions of a peripheral restenotic lesion, illustrating migratory events of an activated SMC. Detail of a thick, partially disrupted internal elastic lamina (IEL) and a metabolically activated SMC crawling through a central gap of the IEL. The disorganized ECM and the fragmented basement membrane indicate proteolytic activity, which allows facilitated motility of the SMC. N = nucleus; RER = rough endoplasmic reticulum; x8,600.

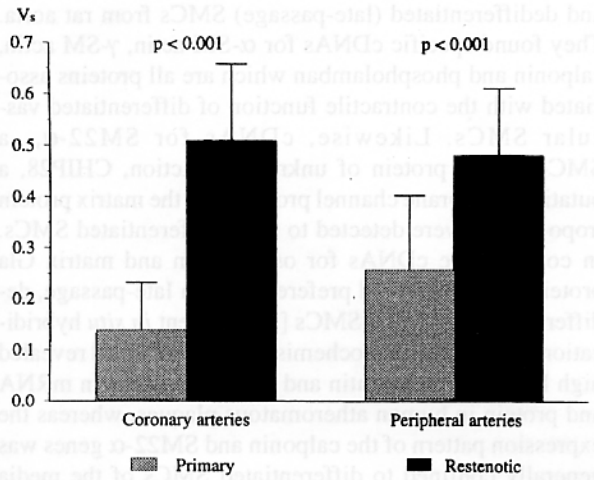

Figure 5. Bar graphs of SMC organelle volume fraction V_s found in coronary and peripheral plaque tissue from primary and restenotic lesions (groups A plus C vs groups B plus D, $p < 0.001$; group A vs group B, $p < 0.001$; group C vs group D, $p < 0.001$).

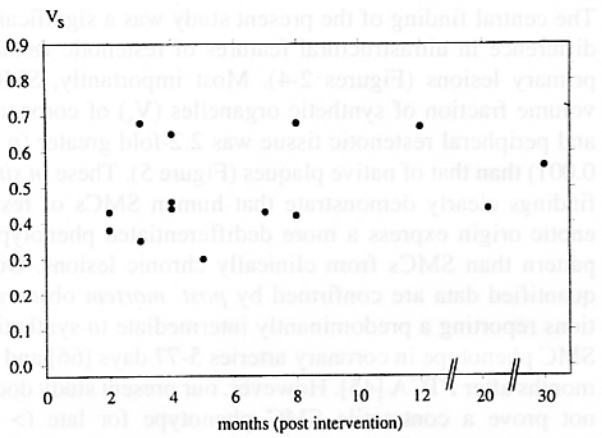

Figure 6. Relationship between SMC organelle volume fraction Vs found in coronary and peripheral restenoses and the time period after angioplasty. Even narrowing the observed time interval below 12 months did not present a significant correlation of Vs to time post intervention.

SMCs. As was commonly seen in numerous atherectomy specimens, a broad basement membrane measuring up to 400 nm and pericellularly surrounding the SMC, was located in the transition territory between cell membrane and extracellular matrix (Figure 2b). Restenotic hypercellular plaques contained closely packed SMCs only partly surrounded by components of the basement membrane. In most cases, extracellular matrix fields were found to be desintegrated and fragmented (Figure 3a). Restenotic SMCs typically presented a cytoplasmic texture that was characterized by a pronounced richness of RER membranes, Golgi apparatus and mitochondria. As illustrated by Figure 3a, SMCs with an excessively high volume fraction of organelles that extended to peripheral cytoplasmic fields were seen, suggesting increased synthetic as well as secretory activity (intermediate to synthetic phenotype SMCs). Moreover, in a few cases with deep atherectomy cutting, when additional portions of the medial layer had been removed, we found these activated SMCs migrating to intimal regions through gaps of the internal elastic lamina (Figure 4). In addition, experimental work with SMCs cultivated from arterial tissue samples revealed intermediate to synthetic phenotype SMCs (Figure 3b) mimicking the low degree of differentiation commonly observed in restenotic plaque tissue.

Quantitative analysis of SMC differentiation - Restenotic versus primary lesions

In order to assess the degree of SMC differentiation and to screen this determinant for a possible relationship to distinct lesions types, SMC phenotype was morphometrically quantitated by the volume fraction of synthetic organelles V_s. Morphometric assessment revealed a broad range of V_s values between 0.02 and 0.60 for primary lesion cells, and between 0.30 and 0.68 for recurrent lesion cells. Most importantly, a significantly larger V_s was seen in restenotic compared to primary plaque SMCs (groups B plus D: 0.49 ± 0.13, n = 14; groups A plus C: 0.22 ± 0.14, n = 27). Detailed V_s analysis of coronary and peripheral arteries showed comparable values for both restenotic groups (group B: 0.51 ± 0.15; n = 5; group D: 0.48 ± 0.13, n = 9). In contrast, means for corresponding native lesion SMCs were found to be reduced (group A: 0.13 ± 0.10, n = 7; group C: 0.25 ± 0.15, n = 20). Figure 5 summarizes the V_s values assessed for all subgroups and emphasizes the clearly different pattern of SMC differentiation between restenotic and primary lesions of both coronary and peripheral arteries. Regarding the restenosis group with increased V_s values, no difference was seen between lesions following balloon angioplasty and those after directional atherectomy (data not shown). Likewise, no trend to V_s mitigation was noticed with prolonged time interval after angioplasty treatment (Figure 6). Interestingly, when arterial SMCs in culture were assessed for phenotype expression, V_s ranged from 0.20 to 0.67 and presented a mean value of 0.44 ± 0.14 (n = 40 SMCs analyzed in seven experiments). These *in vitro* data were closely comparable to V_s values typically found in restenotic tissue ($p = 0.22$), but differed significantly from those characterizing primary plaque SMC phenotype ($p < 0.001$), thereby demonstrating a striking resemblance of isolated to restenotic SMCs in their pattern of decreased differentiation as response to injury.

Discussion

Distinct micromorphologic profiles of restenotic versus primary lesions

This study is based on morphometric analysis of 41 different restenotic and primary arteriosclerotic lesions and is supported by the micromorphologic profiles of SMCs as the predominant intimal cell type (Figures 1-4).

The central finding of the present study was a significant difference in ultrastructural features of restenotic *versus* primary lesions (Figures 2-4). Most importantly, SMC volume fraction of synthetic organelles (V_s) of coronary and peripheral restenotic tissue was 2.2-fold greater ($p < 0.001$) than that of native plaques (Figure 5). These *in situ* findings clearly demonstrate that human SMCs of restenotic origin express a more dedifferentiated phenotype pattern than SMCs from clinically chronic lesions. Our quantified data are confirmed by *post mortem* observations reporting a predominantly intermediate to synthetic SMC phenotype in coronary arteries 5-77 days [66] and 6 months after PTCA [45]. However, our present study does not prove a contractile SMC phenotype for late (> 6 months) restenosis as recently described [45, 66], but demonstrates continuously increased V_s values (Figure 6). Pronounced organelle richness, as illustrated in Figures 3 and 4, suggests high metabolic and secretory activity of different proteins such as collagens, fibronectin, proteoglycans, growth factors or proteases [23, 37, 48, 63], elements which all decisively contribute to the volume expansion of arteriosclerotic lesions, to its maintenance or to continued inflammation. Our findings reveal not a temporal, but rather a chronic expression of SMC metabolic activation in human restenosis.

Interestingly, this report demonstrates large scale SMC phenotype differentiation in 27 primary lesions, as well as a distinct overlap to that seen in the restenosis group. Again, our pooled data quantitating V_s of both coronary and peripheral primary stenoses revealed significantly reduced amounts compared to corresponding restenotic plaques. Variable SMC phenotype expression had already been semiquantitatively estimated by Stary [60, 61]. Remarkably, SMCs rich in rough endoplasmic reticulum were seen mostly in fibroatheromata and their subsequent, complicated Stary V and VIa-c forms [60, 61], reflecting the heterogeneity of SMCs in human plaques. Morphometric analysis in human carotid arteries revealed a high average V_s value (0.48 ± 0.17) for diffuse intimal thickenings Stary IV but, unfortunately, subcellular characteristics of symptomatic target lesions were not examined [42], which would have allowed a comparison with our data. However, one may interprete the surprisingly similar results as bolstering the idea of SMC activation in both human lesion types.

(De)differentiation of SMCs - Markers and proposed mechanisms of regulation

Ultrastructural characteristics of SMCs found in restenotic lesions indicated decreased differentiation and thereby closely resembled the phenotype pattern expressed by human arterial SMCs in culture (Figures 3 and 4). The striking similarity in subcellular morphology, notably in high V_s values (Figure 5), may suggest that cultivated SMCs represent a suitable model to study differentiation aspects of restenosis rather than those of arteriosclerosis. Shanahan *et al.* [58] used the cell culture model to isolate specific markers of both differentiated (freshly dispersed) and dedifferentiated (late-passage) SMCs from rat aorta. They found specific cDNAs for α-SM actin, γ-SM actin, calponin and phospholamban which are all proteins associated with the contractile function of differentiated vascular SMCs. Likewise, cDNAs for SM22-α, a SMC-specific protein of unknown function, CHIP28, a putative membrane channel protein, and the matrix protein tropo-elastin were detected to mark differentiated SMCs. In contrast, the cDNAs for osteopontin and matrix Gla protein were expressed preferentially in late-passage, dedifferentiated vascular SMCs [58]. Recent *in situ* hybridization and immunohistochemistry studies indeed revealed high levels of osteopontin and matrix Gla protein mRNA and protein in human atheromatous plaques, whereas the expression pattern of the calponin and SM22-α genes was generally confined to differentiated SMCs of the media [59].

The sequence of spatiotemporal SMC phenotype differentiation had been assessed using animal and cell culture models [2, 16, 18, 24, 38, 47, 63]. Studies with rabbit carotid arteries revealed reduced neointimal myofilament volume densities two weeks following balloon denudation [38]. In addition, the SMCs of the underlying media had lost contractile elements and showed ultrastructural features of metabolic activation. By six and 18 weeks after injury, phenotype modulation was confirmed for intimal SMCs, whereas a redifferentiation to the contractile phenotype of control arteries occurred in SMCs of the media [38]. Examination of aortic plaques showed that intimal SMCs express a less mature phenotype than medial cells. Intimal SMCs contained decreased amounts of the SMC markers α-SM actin and SM myosin heavy chains, and expressed proteins typical of the dedifferentiated SMC phenotype, such as cytokeratin 8 and A-fibronectin [24]. Experiments with SMCs cultured from intact rat aorta after enzymatic disaggregation showed that in response to *ex vivo* injury, the cellular volume of myofilaments and the amount of α-SM actin mRNA underwent parallel changes [16]. Both parameters transiently decreased on day 5, but returned to original values after confluency. Also, the α-SM actin protein decreased in culture, but then remained reduced irrespective of the proliferative state of the SMCs or cell culture conditions [16]. Taken together, these data suggest decreased SMC differentiation in arteriosclerotic lesions, but also strongly confirm the ultrastructural *in situ* findings of a graduated pattern with distinct dedifferentiation levels for human restenosis and chronic arteriosclerosis (Figures 2-5).

Another intriguing observation of the present study was that restenotic hypercellular plaques consistently comprised loose, extracellular matrix regions and a fragmented, pericellular SMC basement membrane (Figures 3 and 4). These alterations of the SMC microenvironment may be morphologic counterparts of a structural and compositional modulation of extracellular matrix by various proteases, e.g. plasmin, collagenases or stromelysin, predominantly released from macrophages and SMCs [18, 23,

29, 48]. Once the basement membrane is damaged and can no longer function as a natural barrier, SMCs may interact with specific blood-borne and/or locally generated molecules, thereby switching from a contractile to the synthetic phenotype [63]. *In vitro* studies have shown that dedifferentiation of SMCs was induced by specific extracellular matrix components such as tenascin, fibronectin, notably its cell-attachment sequence (Arg-Gly-Asp-Ser), thrombospondin *etc.* [27, 28, 63]. In contrast, the same cells exhibited a differentiated phenotype when reexposed to laminin, reconstituted basement membrane proteins (Matrigel) or collagen III gel [47, 53]. In this context, integrins play a central role in mediating specific interactions between extracellular matrix structures and those of the SMC cytoskeleton [7, 11, 28, 41, 63]. Likewise, exposure of human arterial SMCs to PDGF or EGF revealed a dedifferentiating effect of these mitogens [15, 31, 63], whereas TGF-β1 markedly enhanced differentiation-specific filamentous α-SM actin [15] and inhibited PDGF-induced DNA synthesis and cell multiplication [20]. In summary, there is a growing body of evidence that modulation of SMCs between a differentiated and a dedifferentiated phenotype is regulated by a complex system of proteases, extracellular matrix components, integrins, cytokines and growth factors, many of which are (over)expressed in restenosis as typical form of accelerated human arteriosclerosis [13, 36, 51].

Implications of SMC differentiation - Functional and therapeutic aspects

As outlined in Figure 4, in a few cases with deep tissue removal, we found SMCs crawling through gaps of the internal elastic lamina. Most interestingly, these SMCs exhibited signs of decreased differentiation, and were surrounded by fragmented basement membrane and severely desintegrated extracellular matrix indicating local proteolytic activity. These observations are confirmed by a preliminary report which showed for day 1-6 post PTCA that SMCs migrating through fenestrae of the internal elastic lamina were characterized by a modulation from the contractile to the synthetic phenotype [46]. Recent Boyden chamber experiments demonstrated that directed migration of cultured rat SMCs through a basement membrane barrier required type IV collagenase activity and was readily suppressed by SMC differentiation [48]. A 72-kD type IV collagenase turned out to be the principal matrix metalloproteinase expressed and secreted by dedifferentiated SMCs [48]. Previously, Thyberg and co-workers [63] have clearly shown that phenotype transition to a dedifferentiated state is an essential prerequisite for specific growth factors to induce SMC replication, but not SMC contraction as found for differentiated cells. These data strongly suggest specific micromorphologic profiles exhibited by individual SMCs to be closely associated with proliferative and migratory events leading to intimal hypercellularity as characteristic feature of human restenosis [5-9, 19, 34, 46, 49, 50].

Therapeutic implications of SMC (de)differentiation for restenosis and arteriosclerosis are obvious. Since phenotype modulation is a very early key process after vascular injury, its deliberate counteraction would result in an attenuated capacity of arterial SMCs to pericellular, motogenic and mitogenic stimuli. Inhibition of subsequent SMC migratory and proliferative events is likely to avoid a complex cascade of events [13, 36, 51] ultimately leading to intimal volume expansion with potential clinical consequences. In this context, exogenous as well as endogenous agents that exert differentiation-promoting effects, such as heparin [17, 20, 25, 54], TGF-β [20, 25], reconstituted basement membrane proteins, laminin and collagen [47, 53], may be considered for therapeutic use, possibly by local application. In addition, increased knowledge about the key factors and regulatory processes involved in SMC differentiation could allow the development of new agents/somatic gene therapy that maintain intact vessels or, at least, mitigate their response to injury. Also, this concept might form the basis of prophylactic and therapeutic strategies in the treatment of other clinical forms of accelerated arteriosclerosis.

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