Smooth muscle cells (SMCs) play an important role in the stabilization of atherosclerotic plaques. They contribute to form a firm fibrous cap by synthesizing and assembling fibrillar collagen I, a process that requires the synthesis of fibronectin and activation of integrins. However, extracellular matrix composition changes during the formation of atherosclerotic plaques. Type I and III collagens predominate in the healthy vessel wall, whereas during atherogenesis, fibronectin and type VIII collagen are synthesized by vascular SMCs.1

In advanced plaques, SMCs and macrophages synthesize matrix metalloproteinases which degrade matrix proteins, thereby weakening the fibrous cap.

During lesion development, SMC phenotypes change from a “contractile” to a “synthetic” phenotype.2 It has been shown that inflammatory mediators and oxidized phospholipids induce phenotypic switching in SMCs and consequently also modulate matrix metalloproteinases and collagen production.3 The SMC phenotype that determines migration and proliferation also controls matrix synthesis and degradation.4

Monomeric type I collagen, a byproduct of collagen I breakdown, has been shown to further potentiate an inflammatory SMC phenotype,5 possibly exacerbating the process of collagen turnover. Moreover, induction of apoptosis in SMCs results in decreased cellularity and thus enhanced vulnerability of atherosclerotic plaques. In summary, imbalance toward decreased production and assembly and increased degradation of matrix proteins such as collagens and fibronectin is believed to result in thinning and thus destabilization of the atherosclerotic fibrous cap.6

Collagen Fibrillogenesis: Cell-Versus Non–Cell-Mediated

In the vascular wall, especially in atherosclerotic lesions, collagen I plays an important role in plaque stabilization. It is believed that the balance between collagen I fibrillogenesis and degradation is crucial for providing stability.6 Therefore, it has been speculated that an imbalance in collagen I turnover in the fibrous cap causes the plaque to rupture, which leads to thrombosis and ultimately myocardial infarction and stroke.

Collagen I fibril formation (fibrillogenesis) occurs in the absence of cells as a result of entropy-driven protein self-assembly.8 However, evidence is accumulating that cellular mechanisms may control the generation of the impressive variety of fibril patterns involved in distinct biological functions in different tissues. The assembly of collagen fibrils on the surface of cells is regulated by various collagen-binding proteins, as well as other proteins that directly or indirectly interact with collagen. Cells activate integrins on their surface to determine assembly sites and produce fibronectin and other types of collagens (collagen V) as nucleators to initiate fibrillogenesis.8 Collagen-binding proteins are either secreted or anchored to the cell membrane and include fibromodulin, SPARC, lumican, decorin, matrilin, and discoidin domain receptors DDR1 and -2, to name a few.

Role of Healthy and “Obese” SMCs in Collagen Fibrillogenesis

Destabilization of atherosclerotic plaques is mainly thought to be mediated by increased matrix metalloproteinase production and apoptosis of SMC, resulting in decreased cell numbers and thus decreased synthesis of matrix proteins. Previously, it has been shown that vascular SMC have the ability to orchestrate the assembly of collagen I, through a process involving integrin α2β1 and RhoA activation and fibronectin polymerization.9 Further, fibril assembly on the surface of SMC could be inhibited by antibodies against α2β1 or α5β1, the latter of which prevents fibronectin assembly. The authors also found that collagen fibril formation was dependent on the cytoskeleton, because lysophosphatidic acid-stimulated RhoA was involved in this process. However, it remained unknown whether pathological changes in SMC function would affect their control of collagen I fibrillogenesis.

It has been known for a long time that vascular SMCs, like macrophages, take up and store excess lipids under conditions of hyperlipidemia.10 SMCs express various forms of scavenger receptors to take up low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL). The lipids are stored in lipid droplets which give those SMCs a macrophage foam cell-like appearance.5,11 Interestingly, in addition to scavenger receptors, expression of lipoprotein lipase was demonstrated as a key enzyme that determines the predisposition of SMCs for lipid accumulation.12 However, knowledge about functional consequences of excessive lipid accumulation in SMC has been limited.
In this issue of Circulation Research, Frontini et al.\textsuperscript{13} show that lipid loading of SMCs with either LDL or VLDL drastically decreases their ability to assemble collagen I as well as fibronectin. The authors show that the inability to assemble matrix proteins was caused by a defect in clustering of \(\alpha 5\beta 1\) and the formation of fibrillar adhesion complexes. Phosphorylation of tensin, which is necessary for the formation of fibrillar adhesion complexes, was significantly decreased in lipid laden SMC. Investigation of signaling mechanisms that are known to be involved in formation of adhesion complexes demonstrated that phosphorylation of src (on Tyr418) and FAK (on Tyr397), both of which are necessary for tensin phosphorylation and fibronectin assembly, was inhibited in lipid-laden SMCs. Interestingly, the authors also found that lipid loading changes the localization of activated src, which is restricted to cell edges in “obese” SMCs.

It is interesting to note that lipid loading of SMCs did not alter synthesis of either fibronectin or collagen I. Expression levels of tensin or vinculin also remained unchanged. Moreover, the actin cytoskeleton and formation of vinculin-containing focal adhesions, as well as expression of SMC genes such as SM-\(\alpha\)-actin, h-caldesmon, or calponin h1 were not changed after lipid loading. Together, these results strongly indicate that the inability of lipid-laden SMCs to assemble collagen involves a membrane based signaling defect. Whether an inability to form membrane-based signaling complexes and/or a change in membrane fluidity is causing these defects needs to be explored in more detail. Nevertheless, the results point to a rather specific inactivation of the collagen assembly machinery by lipid loading.

**Questions and Perspectives**

Further investigation is needed to determine how lipid loading inhibits the signaling pathways that lead to the formation of fibrillar adhesion complexes. One possibility, as discussed by the authors, is that accumulating lipid droplets prevent the membrane organization of signaling complexes. This hypothesis is underlined by the fact that in lipid-laden SMCs activated src was confined to the edges of cells, unable to initiate formation of adhesion complexes. Another possibility is that because of cholesterol loading, the membrane structure and fluidity is changed in a way that would prevent the arrangement of adhesion complexes. Indeed there are many examples where changes in membrane fluidity alter SMC function, including ion channel activity and receptor/integrin signaling.

A mechanism that may contribute to altered signaling is increased oxidative stress that has been observed in cells on lipid loading.\textsuperscript{14,15} Activation of both src and FAK have been shown to be drastically altered in conditions of increased oxidative stress, with important consequences for cytoskeletal organization, integrin activation, and cell adhesion.\textsuperscript{16,17} Because lipid loading of cells increases the formation of free radical species, it will be interesting to examine the contribution of changed redox status to the inhibition of signaling that leads to fibrillar adhesion assembly.

In terms of treatment perspectives, it would be important to know whether the ability to assemble collagen can be restored in obese SMCs. The first thing that comes to mind is whether excess lipid can be removed from obese SMCs. As in macrophage foam cells, increasing lipid efflux may restore cellular functions including matrix assembly in SMCs. Indeed, lipid-lowering therapies have been shown to decrease lipid content and consequently increase stability of atherosclerotic plaques. It remains to be shown whether matrix assembly ability of SMCs is restored after lipid lowering. Finally, one could try to directly activate the signaling pathways that lead to organization of fibrillar adhesion complexes to induce collagen I fibrillogenesis. In support of this strategy, the authors show in elegant studies that forced activation of src and FAK can rescue the ability of obese SMCs to assemble fibrinogen and collagen.

In summary, the finding that “obese” (lipid-laden) SMCs lose their ability to assemble collagen I adds another important feature to the complex involvement of SMCs in controlling matrix production and turnover.

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**References**

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