

Fibrillar Collagen Inhibits Cholesterol Biosynthesis in Human Aortic Smooth Muscle Cells

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Objective—Integrin-mediated cell adhesion to type I fibrillar collagen regulates gene and protein expression, whereas little is known of its effect on lipid metabolism. In the present study, we examined the effect of type I fibrillar collagen on cholesterol biosynthesis in human aortic smooth muscle cells (SMCs).

Methods and Results—SMCs were cultured on either fibrillar or monomer collagen for 48 hours and [¹⁴C]-acetate incorporation into cholesterol was evaluated. Fibrillar collagen reduced by 72.9±2.6% cholesterol biosynthesis without affecting cellular cholesterol levels. Fibrillar collagen also reduced 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA) promoter activity (−72.6±7.3%), mRNA (−58.7±6.4%), protein levels (−35.5±8.5%), and enzyme activity (−37.7±2.2%). Intracellular levels of the active form of sterol regulatory element binding proteins (SREBP) 1a was decreased by 60.7±21.7% in SMCs cultured on fibrillar collagen, whereas SREBP2 was not significantly affected (+12.1±7.1%). The overexpression of the active form of SREBP1a rescued the downregulation of fibrillar collagen on HMG-CoA reductase levels. Blocking antibody to α2 integrin partially reversed the downregulation of HMG-CoA reductase mRNA expression. Finally, fibrillar collagen led to an intracellular accumulation of unprenylated Ras.

Conclusions—Our study demonstrated that α2β1 integrin interaction with fibrillar collagen affected the expression of HMG-CoA reductase, which led to the inhibition of cholesterol biosynthesis in human SMCs. (*Arterioscler Thromb Vasc Biol.* 2009;29:1631-1637.)

Key Words: cholesterol ■ integrins ■ HMG-CoA ■ mevalonate ■ Ras

Smooth muscle cells (SMCs) normally reside in the media of the artery surrounded by a meshwork of extracellular matrix (ECM) components, including type I collagen. The adhesion of SMCs to type I collagen is mainly mediated by the interaction with α1β1 and α2β1 integrins that play a pivotal role as a receptor for ECM. After their occupancy and clustering, integrins can activate intracellular signaling pathways,^{1,2} followed by the induction of transcription factors, and subsequent gene and protein expressions.³ Indeed, ECM not only provides a scaffold for mechanical support and tissue organization but also directly alters cell behavior by influencing proliferation, migration, differentiation, and gene expression.^{4,5} To resemble the extracellular environment surrounding the SMCs present in the arterial wall, a cell culture system based on 3 dimensional type I fibrillar collagen gel has been developed.^{3,6} Cells cultured on fibrillar collagen have been also compared to cells cultured on petri dishes coated with monomeric collagen, a structural distinct

form of collagen that has been considered to resemble the ECM environment surrounding SMCs in atherosclerotic plaques.^{4,6,7} In this in vitro system, several changes have been described, from inhibition of cell proliferation,⁷ migration,^{8,9} dedifferentiation,⁴ and ECM remodeling,^{6,8,10} supporting the notion that cell–collagen interaction directly regulates SMC function. Although these studies have helped to understand the role of type I collagen on SMC behavior, very little is known on its effect on cholesterol biosynthesis and on mevalonate (MVA) pathway. On this matter it is important to note that cholesterol present in the plasma membrane lipid rafts has been shown to affect signal transduction events initiated by cell adhesion to the ECM through integrins.^{11,12}

Cholesterol represents one critical end-product of the MVA pathway required for cell membrane formation in proliferating cells.¹³ Moreover, MVA is not only the precursor of cholesterol but also of a number of nonsteroidal

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isoprenoid moieties, including farnesyl-pyrophosphate (F-PP) and geranylgeranyl-pyrophosphate (GG-PP).¹⁴ These 2 isoprenoid derivatives are indeed substrates of prenyltransferases that catalyze the prenylation of several intracellular proteins, including the small GTP-binding protein Ras and Ras-like proteins, such as Rho and Rac involved in several cellular activities.^{3,15–18} Thus, the inhibition of the MVA pathway may impair the activity of several intracellular proteins, resulting in different cellular behavior. It is for this reason that the MVA pathway and thus the cholesterol biosynthesis are tightly regulated cellular events.¹⁹

Sterol regulatory element binding proteins (SREBPs), bound to membranes of the endoplasmic reticulum (ER) and nuclear envelope, play a pivotal role in the regulation of cellular cholesterol content.²⁰ The family consists of 3 different proteins, SREBP1a, SREBP1c, and SREBP2. In cultured cells, SREBP1a is expressed at much higher levels than SREBP1c.²¹ The membrane associated proteins are transcriptionally inactive and are bound to the SREBP-cleavage-activating protein (SCAP), which functions as a sterol sensor.^{22,23} When cells are deprived of sterols, SCAP escorts the SREBPs from the ER to the Golgi, where a 2-step proteolytic cleavage releases the amino terminus portion of the SREBPs from cell membranes. These transcriptionally active fragments of the SREBPs translocate to the nucleus activating the transcription of genes involved in cholesterol biosynthesis and in the MVA pathway.^{24,25}

SREBP1a is a potent activator of all SREBP-responsive genes, including those that mediate the biosynthesis of cholesterol, fatty acids (FA), and triglycerides, but the roles of SREBP1c and SREBP2 are more restricted.²⁶ It is generally assumed that genes involved in FA metabolism (eg, FA-synthase) are preferentially regulated by SREBP1c, whereas the expression of enzymes involved in cholesterol metabolism (eg, HMG-CoA synthase and reductase) and uptake (LDL-receptor) are equally modulated by the activity of both SREBP1c and SREBP2.^{26–28}

In the present study we have investigated the effects of type I fibrillar collagen on cholesterol metabolism in human aortic SMCs. We show that fibrillar collagen, through $\alpha 2\beta 1$ integrin, has a profound effect on HMG-CoA reductase expression, event that altered cholesterol biosynthesis in human aortic SMCs, without changing the intracellular cholesterol content.

Experimental Procedures

Cell Culture and Collagen Matrix Preparation

Human aortic SMCs were isolated from the thoracic aorta of cardiopathic patients undergoing cardiac transplantation. SMCs between passages 5 and 9 were cultured in 0.2% BSA/DMEM on the surface of 3 dimensional fibrillar collagen gels (PureCol, Nutacon BV, Leimuden, NE, 1.0 mg/mL final concentration) and monomer collagen-coated dishes for 48 hours.^{7,29}

For detailed descriptions of the Materials and Methods, please see supplemental materials (available online at <http://atvb.ahajournals.org>).

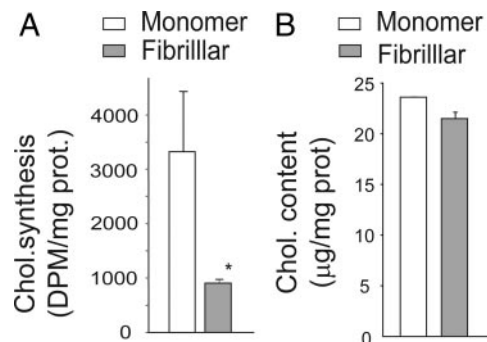


Figure 1. Fibrillar collagen inhibited cholesterol biosynthesis in human SMCs. A, Human SMCs were cultured on monomer and fibrillar collagen in DMEM containing 0.2% BSA in the presence of [¹⁴C]-Acetate. After 48 hours [¹⁴C]-Acetate incorporation into cellular cholesterol was evaluated. Each bar represents the mean \pm SD of triplicate dishes. * $P < 0.01$ fibrillar vs monomer collagen (Student *t* test). The data are representative of 4 replicate experiments. B, Under the same experimental conditions of A, the total intracellular cholesterol content was evaluated by Gas Chromatography. The data are representative of 2 replicate experiments.

Results

Fibrillar Collagen Inhibits Cholesterol Biosynthesis in Human SMCs

To study the effect of cell–collagen interaction on cholesterol homeostasis we examined the effects of structurally distinct forms of type I collagen, monomer versus polymerized fibrils. All the experiments were performed under serum free conditions, to induce cholesterol biosynthesis and to avoid any confounding factors deriving from growth factors and cytokines present in the FCS. Compared to monomer, fibrillar collagen reduced cholesterol biosynthesis, measured by incorporation of [¹⁴C]-acetate into total cholesterol, by $72.9 \pm 2.6\%$ (Figure 1A). In contrast, the total intracellular cholesterol content was similar under the two experimental conditions (Figure 1B).

HMG-CoA Reductase Activity Is Responsible for the Reduction of Cholesterol Biosynthesis in Human SMCs Cultured on Fibrillar Collagen

To investigate the molecular mechanisms underlying the inhibition of cholesterol biosynthesis by fibrillar collagen, we measured the HMG-CoA reductase activity, the rate limiting step of the MVA pathway, under the aforementioned experimental conditions.¹³ The conversion of radio-labeled HMG-CoA by isolated microsomes from human SMCs cultured on fibrillar collagen to MVA was reduced by $37.7 \pm 2.2\%$ (Figure 2A). This effect strongly correlated with the expression of HMG-CoA reductase measured by Western blot analysis, which was decreased by $35.5 \pm 8.5\%$ (Figure 2B).

The expression of HMG-CoA reductase is primarily regulated at the transcription levels,¹³ thus we measured the mRNA levels and the promoter activities. As shown in Figure 2C, fibrillar collagen significantly reduced the mRNA of HMG-CoA reductase by $58.7 \pm 6.4\%$, determined by quantitative RT-PCR reaction. The analysis of the HMG-CoA

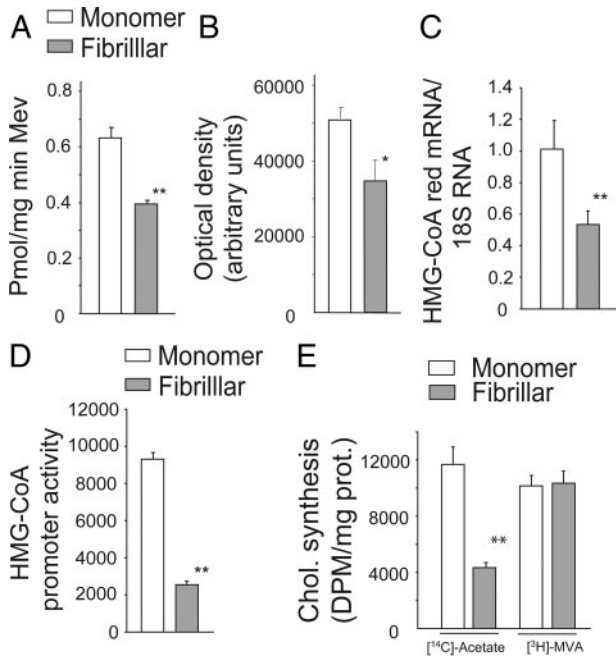


Figure 2. HMG-CoA reductase activity was suppressed in human SMCs cultured on fibrillar collagen. A through E, Human SMCs were cultured for 48 hours with DMEM containing 0.2% BSA on monomer and fibrillar collagen. A, After this period, the HMG-CoA reductase activity was determined by measuring the rate of conversion of radioactive HMG-CoA into MVA in detergent-solubilized cell-free extract as described in Material and Methods. Each bar represents the mean \pm SD of triplicate samples. B, HMG-CoA reductase expression was evaluated by Western blotting analysis of total protein extracts. Quantitative densitometric analysis was performed with Gel Doc acquisition system and Quantity One software (BIO-RAD). C, HMG-CoA reductase mRNA expression was evaluated by quantitative real-time polymerase chain reaction from total RNA. The data are representative of 4 independent experiments. D, HMG-CoA promoter activity was evaluated in human SMCs retrovirally infected with pBM-SIN-HMG-CoApro-luciferase construct. The data are representative of 2 independent experiments. E, Human SMCs were cultured on monomer and fibrillar collagen in DMEM containing 0.2% BSA in the presence of either [¹⁴C]-Acetate or [³H]-MVA. After 48 hours [¹⁴C]-Acetate and [³H]-MVA incorporation into cellular cholesterol was evaluated. Each bar represents the mean \pm SD of triplicate dishes. The data are representative of 2 independent experiments. **P* < 0.05; ***P* < 0.01 by Student *t* test.

reductase promoter activity by a luciferase assay also showed a significant 72.6 \pm 7.3% reduction in human SMCs cultured for 48 hours on fibrillar collagen, compared to monomer (Figure 2D).

To directly demonstrate that the inhibition of cholesterol biosynthesis elicited by fibrillar collagen was dependent by the activity of the HMG-CoA reductase, we measured the incorporation of radiolabeled MVA, the product of HMG-CoA reductase reaction, into cellular cholesterol.¹³ As shown in Figure 2E, the evaluation of cholesterol biosynthesis by incubating SMCs with [¹⁴C]-acetate was strongly suppressed by fibrillar collagen, whereas the incorporation of [³H]-MVA was not affected.

Taken together, fibrillar collagen significantly affected cholesterol biosynthesis by inhibiting the transcription and therefore the activity of HMG-CoA reductase, the rate-limiting step of the MVA pathway.

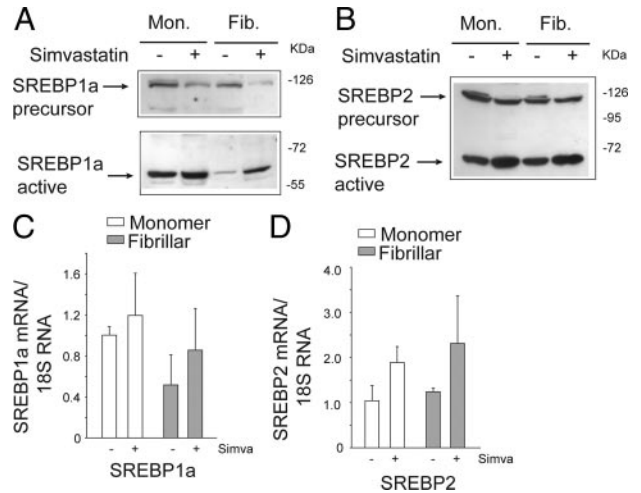


Figure 3. Fibrillar collagen affected SREBP1a activation in human SMCs. A through D, Human SMCs were cultured for 48 hours with DMEM containing 0.2% BSA on monomer and fibrillar collagen. Two hours before preparation of protein extracts 25 μ g/mL of the proteasome inhibitor ALLN was added to the cultured media. SREBP1a and SREBP2 expression and processing were then evaluated by Western blotting analysis of total protein extracts (A and B). As a positive control, cells were incubated with simvastatin (5 μ mol/L). For SREBP1a precursor form the film was exposed for 10 minutes, whereas for SREBP1 active form for 2 minutes. C and D, mRNA expression levels of SREBP1 and SREBP2 were evaluated by quantitative real-time polymerase chain reaction from total RNA extracted from SMCs cultured for 24 hours on monomer and polymerized collagen.

Fibrillar Collagen Regulates SREBP-1a Transcriptional Activity

Several studies have firmly established the pivotal role of SREBPs in the regulation of HMG-CoA reductase transcription.^{13,28} To investigate whether the observed reduction of HMG-CoA reductase expression levels mediated by fibrillar collagen was dependent by the transcriptional activity of SREBPs, we evaluated the processing and the expression of SREBP1a and SREBP2. Western blotting analysis from total cell lysates demonstrated that fibrillar collagen reduced the expression levels of the active form of SREBP1a at 68 kDa (-60.7 \pm 21.7%) with a minor reduction of the pro form at 125 kDa (-20.4 \pm 9.2%; Figure 3A). In contrast, the expression of the active form of SREBP2 was not dramatically altered by fibrillar collagen compared to monomer (+12.1 \pm 7.1%) (Figure 3B). In addition, the incubation with simvastatin induced both SREBP1a and SREBP2 processing in both SMCs cultured on monomer and fibrillar collagen (Figure 3A and 3B).

The expression of levels SREBP1a and SREBP2 mRNA were also measured by RT-PCR from total RNA extracted from SMCs cultured for 24 hours on monomer and fibrillar collagen. Fibrillar collagen reduced by 48.3 \pm 29.4% the expression level of SREBP1 mRNA, although this difference was found not to be statistically significant (Figure 3C), whereas SREBP2 expression levels were found similar between monomer and fibrillar collagen (Figure 3D). The incubation with simvastatin induced both genes in SMCs cultured either on monomer or fibrillar collagen (Figure 3C and 3D).

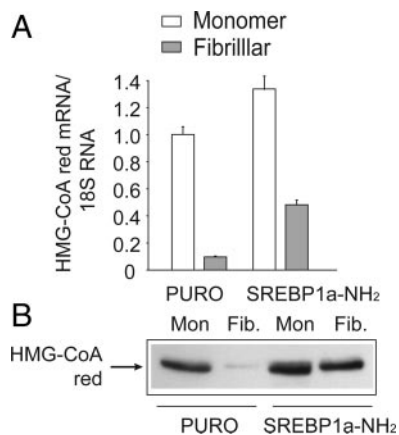


Figure 4. Overexpression of SREBP1a active form rescued the effect of fibrillar collagen on the expression of HMG-CoA reductase. A and B, SMCs were transduced with pBM-IRES-PURO retrovirus encoding control vector (PURO) or with SREBP1aNH₂-IRES-PURO encoding the active form of SREBP1 and then cultured on monomer and fibrillar collagen for 48 hours in DMEM containing 0.2% BSA. HMG-CoA reductase mRNA (A) and protein expression (B) were evaluated by quantitative real-time polymerase chain reaction from total RNA and by Western blotting analysis for total protein extracts, respectively.

To further establish the effect of fibrillar collagen on SREBP1a, we analyzed the mRNA levels of two genes directly regulated by this transcription factor, such as HMG-CoA synthase and FA-synthase.²⁸ The expression levels of HMG-CoA synthase and FA-synthase in SMCs cultured on fibrillar collagen were reduced by $46.2 \pm 6.4\%$ and $67.3 \pm 16.0\%$, respectively, further supporting the effect of fibrillar collagen on SREBP1a activity (supplemental Figure I).

To directly demonstrate that SREBP1 transcriptional activity was responsible for the reduction of HMG-CoA reductase in SMCs cultured on fibrillar collagen, we retrovirally overexpressed in SMCs the active amino terminal portion of SREBP1a. As shown in Figure 4, the overexpression of

SREBP1a in SMCs led to the upregulation of HMG-CoA reductase mRNA (Figure 4A) and protein (Figure 4B) in SMCs on either monomer or fibrillar collagen, compared to cells transduced with empty vector control. Finally, the downregulation of SREBP1 in human cultured SMCs by two different siRNA probes significantly reduced the expression of HMG-CoA reductase on both monomer and fibrillar collagen (supplemental Figure II).

Taken together these results demonstrate that the inhibition of HMG-CoA reductase transcription observed in human SMCs cultured on fibrillar collagen is dependent by the activity of SREBP1a.

$\alpha 2\beta 1$ Integrin Collagen Receptor Mediates the Reduction of HMG-CoA Reductase Transcription

The adhesion of SMCs to fibrillar collagen is primarily mediated by $\alpha 1\beta 1$ and $\alpha 2\beta 1$ integrin receptors.³⁰ To investigate which of these two collagen receptors were responsible for the observed responses, we first determine the effect of blocking antibodies toward $\alpha 1$ and $\alpha 2$ integrin subunits on SMC adhesion to fibrillar collagen. In agreement with previous studies,³¹ the adhesion of human SMC to collagen is largely mediated by $\alpha 2\beta 1$ integrin, indeed the anti- $\alpha 2$ blocking antibody reduced cell adhesion by $70.0 \pm 3.2\%$, whereas anti- $\alpha 1$ antibody affected only the $27.4 \pm 5.1\%$ (Figure 5A). Interestingly, the mRNA expression levels of $\alpha 2$ and $\beta 1$ integrin subunits in SMCs cultured for 48 hours on fibrillar collagen were increased by 8.9- and 8.7-fold, respectively, whereas mRNA of $\alpha 1$ subunit was unaffected (Figure 5B).

Incubation of human SMCs with blocking antibody to $\alpha 2$ integrin significantly prevented the effect of fibrillar collagen on HMG-CoA reductase mRNA expression, whereas a blocking antibody directed to the $\alpha 1$ integrin subunit was ineffective (Figure 5C). These data indicate that the interaction of SMCs with fibrillar collagen through $\alpha 2\beta 1$ integrin partially

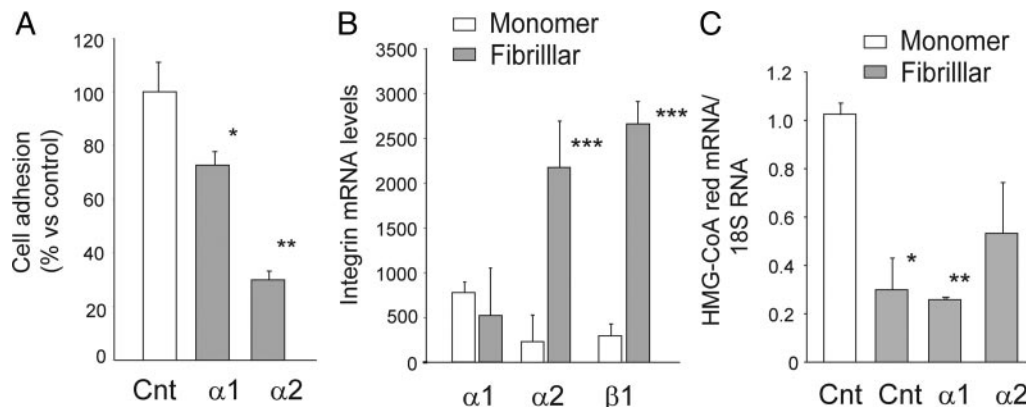


Figure 5. Blocking antibody against $\alpha 2$ integrin abrogated the downregulation of HMG-CoA mRNA levels elicited by fibrillar collagen. A, SMCs were incubated in suspension for 30 minutes with either $\alpha 1$ or $\alpha 2$ blocking antibody, and then the adhesion to fibrillar collagen was measured as described in Materials and Methods. B, $\alpha 1$, $\alpha 2$, and $\beta 1$ integrin subunit mRNA levels were determined from total RNA isolated from human SMCs cultured for 48 hours in DMEM containing 0.2% BSA by GEArray Microarrays for Extracellular Matrix & Adhesion Molecules Arrays (SABioscience) according to manufacturer's instructions. C, Human SMCs were incubated in suspension for 30 minutes with control IgG, anti- $\alpha 1$, and anti- $\alpha 2$ blocking antibody and then cultured on monomer and fibrillar collagen in DMEM containing 0.2% BSA. After 48 hours HMG-CoA reductase mRNA levels were determined by quantitative real-time polymerase chain reaction from total RNA. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ fibrillar vs monomer collagen (Student *t* test). The data are representative of 3 independent experiments.

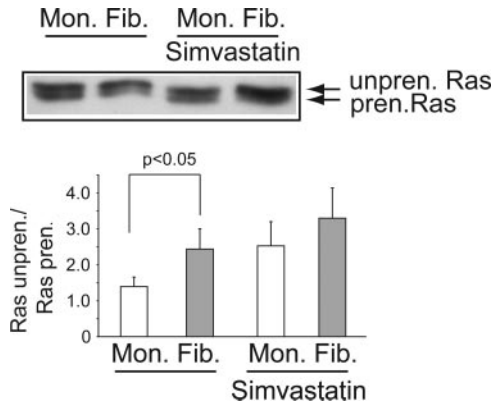


Figure 6. Fibrillar collagen reduced Ras farnesylation in human SMCs. Total cell lysates were prepared from human SMCs cultured on monomer and fibrillar collagen for 48 hours in DMEM containing 0.2% BSA. As a control, total protein extract was prepared from human SMCs incubated with 5 $\mu\text{mol/L}$ simvastatin. Ras prenylation was evaluated by Western blotting analysis with a specific antibody anti-Ras. The slower migrating band represents the unprenylated form of Ras (unpren. Ras), whereas the faster migrating band is prenylated Ras (pren. Ras). Lower panel, Quantitative densitometric analysis of prenylated and unprenylated Ras was performed with Gel Doc acquisition system Quantity One software (BIO-RAD) from films of 3 independent experiments.

mediates the downregulation of HMG-CoA reductase and cholesterol biosynthesis.

Ras Farnesylation Is Partially Impaired in Human SMCs Cultured on Fibrillar Collagen

Because the HMG-CoA reductase activity is the rate-limiting step of the MVA pathway, the inhibition of its catalytic activity in human SMCs could affect the biosynthesis of isoprenoids involved in protein prenylation, such as F-PP and GG-PP. Thus, to study whether fibrillar collagen can alter the prenylation of intracellular proteins we determined, by Western blotting analysis, the expression of unprenylated and prenylated forms of Ras, a pivotal regulator of cell proliferation. As shown in Figure 6, in human SMCs cultured on fibrillar collagen a significant accumulation of unprenylated form of Ras was observed. As expected, similar results were obtained with simvastatin.³²

Discussion

In the present study we showed, for the first time, that adhesion of human aortic SMCs on fibrillar collagen strongly impaired cholesterol biosynthesis, the major end-point product of the MVA pathway. Because the HMG-CoA reductase is the rate limiting step of the MVA pathway,¹³ we focused our attention on this enzyme by determining the effect of fibrillar collagen at four different levels: the promoter activity, the mRNA and protein expression, and the enzyme activity. All these parameters were significantly inhibited by fibrillar collagen, indicating that the observed reduction of cholesterol biosynthesis was indeed the result of reduced HMG-CoA reductase activity. A direct demonstration that the

activity of this enzyme was affected by fibrillar collagen was then provided by the fact that the incorporation of radiolabeled MVA, the product of HMG-CoA reductase reaction, into cellular cholesterol was not affected. Together with the HMG-CoA reductase we also found a significant reduction of the expression of the HMG-CoA synthase mRNA level, indicating that both enzymes contributed to the inhibition of the cholesterol biosynthesis in SMCs cultured on fibrillar collagen.

Many of the mRNAs encoding enzymes involved in cholesterol and FA synthesis are coordinately regulated by the SREBP family of membrane-bound transcription factors.^{26,28} We therefore determined whether SREBPs were involved in the regulation of HMG-CoA reductase expression by fibrillar collagen. We observed that SREBP1a, but not SREBP2, was differentially processed in cells cultured on fibrillar versus monomer collagen. In particular, we observed a consistent reduction of the active SREBP1a cleaved product in human SMCs cultured on fibrillar collagen and a minor reduction of the pro form of SREBP1a. In association with these changes the inhibition of SREBP1a transcriptional activity was demonstrated by the fact that the HMG-CoA reductase promoter activity and the mRNA expression levels of two genes directly regulated by SREBP1a (ie, FA-synthase and HMG-CoA synthase^{27,28}) were reduced in SMCs cultured on fibrillar collagen.

The direct relationship between SREBP1a transcriptional activity and the expression of HMG-CoA reductase was also demonstrated by the fact that the exogenous overexpression of the active form of SREBP1a almost completely prevented the effect of fibrillar collagen on HMG-CoA reductase levels at the protein and mRNA levels. Moreover, a partial reduction of SREBP1a levels by siRNA significantly reduced the expression of HMG-CoA reductase mRNA.

Although we clearly demonstrated a reduction of SREBP1a transcriptional activity in SMCs on fibrillar collagen, the molecular mechanism governing this event still needs to be determined. The fact that the mRNA levels of SREBP1a was reduced, but not in a statistically significant manner, in response to fibrillar collagen indicates that the regulation of SREBP1a may partially occur at the transcriptional levels, but additional posttranscriptional regulation mechanisms cannot be excluded. Further experiments will be carried out to determine the effect of fibrillar collagen on SREBP transport from ER to the Golgi, a very complex event regulated by several factors, such as SCAP, Site-1, and Site-2 protease, and Insig1 and Insig2.^{26,33}

The activation of SREBPs is a tightly regulated process that responds primarily by the intracellular concentrations of sterols.¹⁹ In the present study all the experiments were performed in serum-free media, a condition that induces the cholesterol neosynthesis by the activation of SREBP1a and SREBP2.^{19,34} Under these conditions we did not observe any significant change of total intracellular cholesterol content, suggesting a specific role of fibrillar collagen in the regulation of SREBP1a-dependent HMG-CoA reductase expression. The lack of reduction in total cholesterol content was expected for at least 3 reasons: (1) the incubation time used was relatively short (48 hours),

(2) the cultured media used was free of cholesterol acceptors (ie, HDL), and (3) the cells were in a quiescent state under both cultured conditions.^{6,7} Moreover, our observation is in agreement with pioneer experiments conducted by Maltese et al³⁵ in which the suppression of cholesterol biosynthesis by 25-hydroxycholesterol in C-6 glioma cells incubated with serum free media for 48 hours did not affect the sterol/protein ratio.

The interaction of human SMCs to collagen is primarily mediated by $\alpha 1\beta 1$ and $\alpha 2\beta 1$ integrin receptors. By gene array analysis we confirmed a previous observation that fibrillar collagen specifically induces $\alpha 2$ and $\beta 1$ integrin subunit in human SMCs.^{3,18} By using blocking antibodies to $\alpha 1$ and $\alpha 2$ integrin subunits we then demonstrated that the $\alpha 2\beta 1$ integrin receptor is at least partially involved in the suppression of HMG-CoA reductase mediated by fibrillar collagen, whereas $\alpha 1\beta 1$ had no effect. These data indicate that other collagen receptors may be involved in this cellular response, including the recently discovered Discoidin domain receptors (DDR).^{29,36}

Importantly, cell signal transduction events initiated by cell adhesion to the ECM through integrins are profoundly regulated by the presence of cholesterol within the plasma membrane lipid rafts.^{11,12} Indeed, disruption of lipid rafts by cholesterol depletion mimicked loss of cell adhesion, preventing targeting of prenylated protein Rac to the plasma membrane and its coupling with downstream effector molecules.^{11,37} Reports from Pozo et al and Palazzo et al also indicate that lipid rafts are commandeered by integrins to target Rho and Rac to the cell membrane.^{11,12} Thus, integrin-mediated adhesion to ECM appears to be directly involved in lipid raft assembly. Moreover, an elegant study conducted in $\beta 1$ null fibroblast cell line demonstrated that cell adhesion through $\beta 1$ integrin receptor regulates the amount of cholesterol present in the lipid raft without altering the total intracellular cholesterol levels.³⁸ Our study further implements these observations demonstrating, for the first time, that signaling from integrin receptors regulates cholesterol biosynthesis by altering the activity of HMG-CoA reductase. Moreover, we demonstrated that the inhibition of HMG-CoA reductase and therefore the MVA pathway affected protein prenylation of Ras. On this matter it is tempting to speculate that the inhibition of Ras prenylation, and therefore its function, may contribute to the G1 arrest of the cell cycle described in human SMCs cultured on fibrillar collagen.⁷

The effect of fibrillar collagen on SMC proliferation has been previously interpreted to being relevant in maintaining SMCs in a low proliferative index in normal media, whereas in response to proteolytic enzymes, the degradation of collagen may alter the cellular response to growth factors facilitating the accumulation of SMCs within the atherosclerotic plaque.^{6,7} Our findings further implement this hypothesis demonstrating that the suppression of the MVA pathway and protein prenylation may be one of the basic molecular events implicated in the maintenance of SMCs in a quiescent state

and preventing their accumulation in the neointimal during atherogenesis.

In association with cell cycle arrest Koyama et al have described a reduction of focal adhesion sites and actin stress fibers formation in SMCs cultured on fibrillar collagen.⁷ These effects may be indicative of a deregulation of the prenylated proteins Rho, Rac, and Cdc42, potentially related to the reduced activity of HMG-CoA reductase and therefore protein prenylation.³⁹ Further studies will be aimed at investigating the effect of fibrillar collagen on Rho protein function as related to cell morphology and plasma lipid rafts formation.

In conclusion, our study adds new insights in the complexity of the regulation of cholesterol homeostasis, revealing the $\alpha 2\beta 1$ integrin-dependent effect of fibrillar collagen on HMG-CoA reductase expression and synthesis of MVA-derived isoprenoids involved in protein prenylation processes. Type I fibrillar collagen-dependent regulation of HMG-CoA reductase expression reported here could perhaps be informative in understanding the basic molecular mechanisms that regulate SMC accumulation in atherosclerotic plaque but also for the development of dyslipidemia in patients affected by liver fibrosis where deposition of fibrillar collagen is observed.^{40,41}

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Disclosures

None.

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