Mechanical Stretch-Induced Apoptosis in Smooth Muscle Cells Is Mediated by β_1-Integrin Signaling Pathways

Florian Wernig, Manuel Mayr, Qingbo Xu

Abstract—Recently we demonstrated that mechanical stress induces apoptosis of vascular smooth muscle cells in vitro and in vein grafts (Mayr et al. *FASEB J.* 2000;15:261–270). The current study was designed to investigate molecular mechanisms of mechanical stretch–induced apoptosis. Smooth muscle cells cultivated on silicone elastomer plates precoated with collagen I, elastin, laminin, or Pronectin were subjected to cyclic mechanical stretch. Interestingly, in response to mechanical stress, the number of apoptotic cells increased significantly in cells growing on collagen I–coated plates but not on other matrixes. We therefore thought that receptors mediating binding to collagen I, such as integrin β_1 containing receptors, might be involved in signaling pathways leading to stretch-induced apoptosis. On collagen plates, mechanical stress rapidly activated p38 MAPK that phosphorylated p53 in smooth muscle cells. Lack of functional *Rac* completely abrogated p38 MAPK-p53 activation as well as apoptosis. Furthermore, mechanical stress resulted in increases of both integrin β_1 protein expression and activity as identified by Western blotting and *Shc* immunoprecipitation assays. Treatment with a β_1-integrin–blocking antibody or integrin signaling inhibitor cytochalasin B but not growth factor receptor inhibitor suramin abrogated both stretch-induced phosphorylation of p38 MAPK and p53 expression. Akin to the inhibition of p38 MAPK-p53 signaling, pretreatment with a β_1-integrin–blocking antibody or cytochalasin B but not suramin inhibited stretch-induced apoptosis on collagen plates. These results suggest that mechanical stress–induced apoptosis in vascular smooth muscle cells is mediated by β_1-integrin–*rac*–p38-p53 signaling pathways. (*Hypertension*. 2003;41:903-911.)

Key Words: apoptosis ■ integrins ■ signal transduction ■ muscle, smooth ■ collagen

Blood vessels are dynamically subjected to mechanical forces in the form of stretch and shear stress resulting from blood pressure and blood flow, respectively. Whereas shear stress is mainly sensed by endothelial cells that line the vessel, stretch stress affects all cell types in the vessel wall.[1] In vivo, various factors ranging from physical exertion to psychological stress lead to a transient rise in blood pressure, and if the factors are persistent and chronic, arteriole walls gradually thicken, resulting in chronic hypertension.[2,3] Large arteries, such as the aorta, coronary, and carotid arteries, undergo adaptation or remodeling in response to elevated blood pressure leading to arterial hypertrophy and/or hyperplasia, that is, atherosclerosis.[4,5] Therefore, mechanical stress could be a crucial factor in the pathogenesis of hypertension-induced atherosclerosis.[6]

In recent years, apoptosis of vascular smooth muscle cells (SMCs) has been increasingly implicated in both development and outcome of atherosclerotic disease.[7–10] We demonstrated that SMC apoptosis occurs at a very early stage in the development of vein bypass atherosclerosis, in which biomechanical stress plays a crucial pathogenetic role.[11] When SMC apoptosis was altered as the result of gene deletions of p53 or protein kinase C δ (PKCδ), increased atherosclerotic lesions were seen in $p53^{-/-}$[12] and $PKC\delta^{-/-}$ mice.[13] Thus, mechanical stress–induced cell death is crucial in the development of atherosclerosis.[14,15]

Integrins are cell surface receptors composed of α- and β-subunits. Each $\alpha\beta$ combination has its own ligand specificity and signaling properties.[16] Integrins enable cell adhesion (cell–matrix, cell–cell) and transduce both chemical and mechanical signals.[17] Certain integrins, together with other receptors and mitogenic factors, have been reported to mediate mechanical stress–induced proliferation in SMCs.[18,19] In cultured endothelial cells, shear stress activated extracellular regulated-protein kinases (ERKs) and c-*Jun* kinases (JNKs),[20] and integrins may function as mechanotransducers for the kinase activation.[20,21] However, no data are available concerning the involvement of integrin-mediated signal transduction pathways leading to cell apoptosis induced by mechanical stress.

We hypothesized that integrins could serve as mechanosensors to convert a physical stimulus into a biological signal, which activates downstream signal transducers leading to apoptosis. In this report, we provide the first evidence that β_1-integrin–containing receptors are transducing stretch signals leading to p53-dependent apoptosis in SMCs through activation of *rac* and p38 MAPK.

Methods

Cells

SMCs were isolated and cultured as described previously.[22,23] Experiments were conducted on SMCs (<20 passages) that had just achieved subconfluence.

Stable Transfection

Rat SMCs were stably transfected with *Ras* N17, *Rac* N17, as described previously.[24] These transfected SMC lines express SMC markers, including SM22, α-actin, calponin, and smooth muscle myosin heavy chain at levels similar to vector-transfected cells (data not shown).

Cyclic Strain

SMC were plated on silicone elastomer–bottomed culture plates precoated with different extracellular matrix (ECM) proteins (Flexcell) at 0.3×10^5 cells per well, grown for 2 days with 15% FCS, and subjected to cyclic strain with a Cyclic Stress Unit (FX4000 AFC-CTL, Flexcell). Cyclic deformation (60 cycles/min) and 7% or 15% elongation were applied. Unless otherwise specified, collagen I–precoated plates were used.

Annexin V and Propidium Iodide Double Staining and FACS Analysis

Annexin V labeling and FACS analysis were performed as described previously.[11]

TUNEL Assay

Accumulated internucleosomal DNA fragments (apoptosis) were detected as described previously.[11,25] Percentages of positive stained cells were determined by counting the numbers of labeled and total cells (counterstaining of cell nuclei with Hoechst 33258). Positive and total cells of 3 regions of each sample were counted.

Analysis of Protein Expression

Western blots were performed in a manner similar to the method described previously, whereby modified RIPA buffer was used for lysis (50 mmol/L Tris-HCl, pH 7.4, 1% NP-40, 0.25% Na-deoxycholate, 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L PMSF, 1 μg/mL aprotinin, leupeptin, and pepstatin, 1 mmol/L Na_3VO_4, 1 mmol/L NaF).[24] Blots were probed with antibodies against p53 (NCL-p53-CM5p, Novocastra), integrin β_1, integrin α_2, pan- or phosphorylated p38 MAPK, phosphorylated ERK 1/2, and MDM2 (Santa Cruz Biotech). For all phosphorylation experiments, SMCs were serum-starved for 48 hours.

Immunoprecipitation

For coimmunoprecipitation of *Shc*-integrin β_1 complexes, cell extracts containing 500 μg of protein were preincubated with normal mouse IgG-agarose (Santa Cruz Biotech). Samples were incubated with 10 μL of antibody against *Shc* (Santa Cruz Biotech) for 1 hour at 4°C. Subsequently, 60 μL of protein G Plus-Agarose suspension (Santa Cruz Biotech) was added. Western blotting was performed using an antibody against integrin β_1. Similarly, platelet-derived growth factor (PDGF) receptor-α was immunoprecipitated with the use of the specific antibody (Santa Cruz Biotech), and Western blotting was performed with the use of antiphosphotyrosine antibody (Upstate Biotechnology).[26]

Kinase Assay

For kinase assays, the procedure used was similar to that described previously.[27,28] p38 MAPK activity in the immunocomplexes was measured by using glutathione *S*-transferase-p53 as substrate (GST-p53, the plasmid was provided by Dr J.Y. Shyy, Department of Bioengineering, University of California), produced in competent cells and isolated with gluthatione-sepharose 4B Redi Pack Columns (Pharmacia Biotech Inc) according to the manufacturer's protocol. The p38 MAPK assay was performed as described previously.[29] The specificity of the band was confirmed by use of myelin basic protein as a substrate.

Statistical Analysis

ANOVA was performed for multiple comparisons. A paired Student t test was used to assess differences between 2 groups. Results are given as mean±SEM. A probability value <0.05 was considered significant.

Results

ECM Modulates Mechanical Stretch–Induced Apoptosis of SMCs

To determine the distinctive role of ECM proteins and integrins in mechanotransduction, SMCs were cultivated on different ECMs and subjected to mechanical stress. Apoptosis was assessed by double staining with annexin V and propidium iodide and FACS analysis. Representative results of FACS analysis are shown in Figure 1A. Interestingly, a significant increase in annexin V–positive cells could only be observed when cells were cultivated on a collagen I matrix but not on elastin, laminin, and Pronectin (Figure 1B). Similarly, SMCs positive for both Annexin V and PI, indicating a later stage of apoptosis, only increased on the collagen I matrix but not on other matrixes (Figure 1A). The TUNEL method was used to confirm the presence of apoptotic nuclei after mechanical stretch (Figure 1C). A significant increase in TUNEL-positive nuclei was present in SMCs on collagen I (Figure 1C) but not on other matrixes (data not shown).

Stretch-Induced Apoptosis Signaling Involves Activation of p38 MAPK and p53

Based on our previous observations,[11,12] we wondered whether p38 MAPK and p53 are also essential parts of the signaling pathway leading to mechanical stress–induced SMC apoptosis on a collagen I matrix. Both stretch-induced phosphorylation of p38 MAPK and expression of p53 protein were induced in stretched SMCs on a collagen I matrix (Figure 2A). However, no significant change in p53 expression was detected on other matrixes (data not shown). Mechanical stress–activated p38 MAPK (Figure 2B) directly phosphorylates p53. Phosphorylation of GST-p53 by p38 MAPK isolated from mechanically stressed SMCs was maximal after 10 minutes (Figure 2C), which was in parallel with mechanical stress–induced p38 MAPK phosphorylation, as shown in Figure 2B.

Small G protein Rac Mediates Stretch-Initiated p38 and p53 Activation and Apoptosis

With the use of SMC stably transfected with plasmid-expressing dominant negative *Ras* (*Ras* N17) or a *myc*-tagged form of a dominant negative *Rac1* (*Rac1* N17), we found that lack of functional *Rac* completely abrogated mechanical
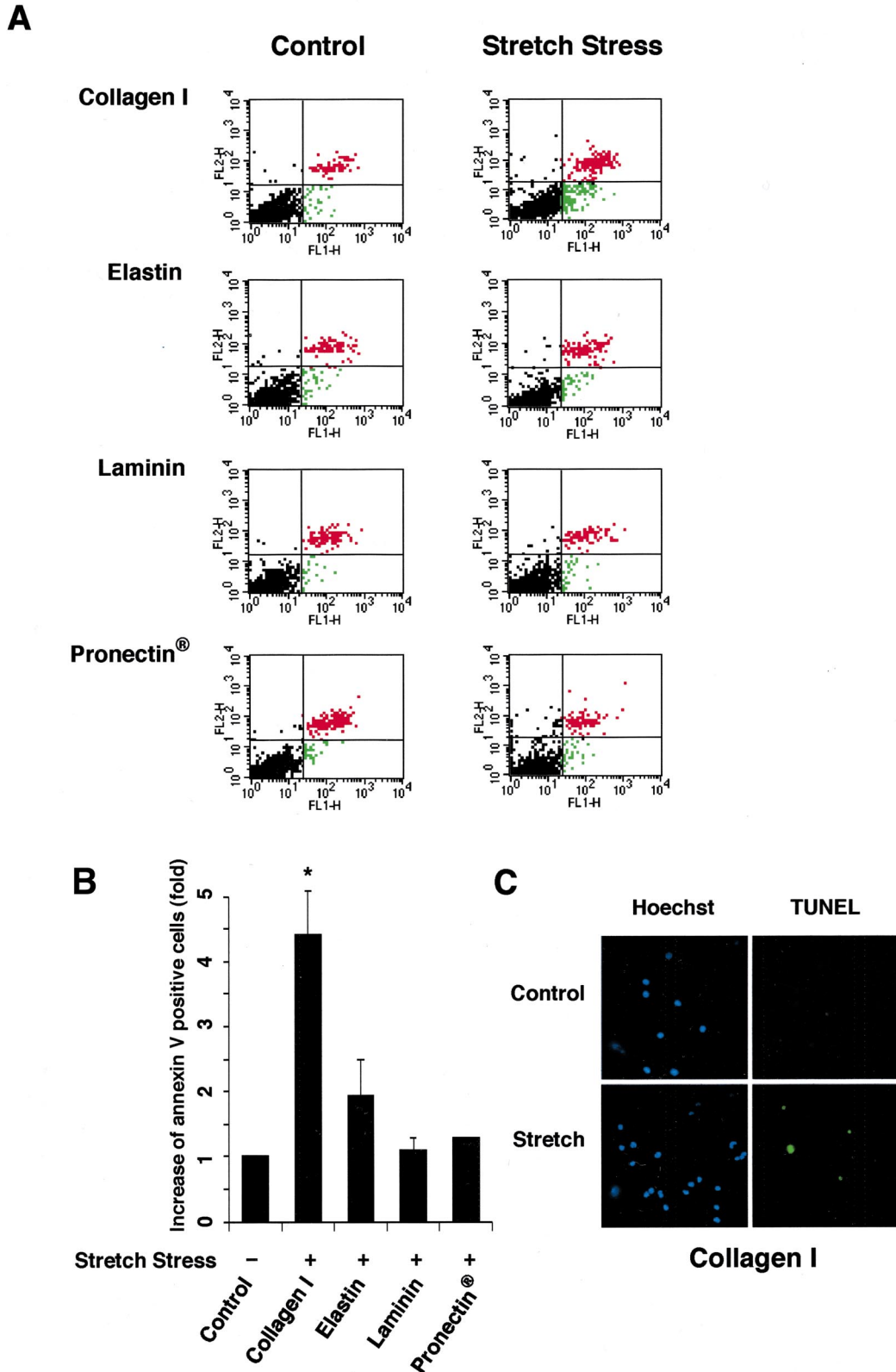
Figure 1. Mechanical stretch–induced apoptosis of SMCs is matrix-dependent. Rat aortic SMCs cultivated on flexible silicone elastomer strain plates precoated with different ECMs were subjected to cyclic mechanical stress for 6 hours. Amount of apoptosis was assessed by double staining with annexin V and propidium iodide and FACS analysis (A, B). A, Typical fluorescence-1/fluorescence-2 profile of stretch-stressed SMCs stained with annexin V and PI. B, Bar graph represents mean values (±SEM) of 3 independent experiments and shows relative increase of annexin V–positive cells compared with unstressed controls. C, Representative areas of unstressed controls and stretch-stressed SMCs stained with a TUNEL kit (green fluorescence) and counterstained with Hoechst 33342 (blue fluorescence). *Significant difference compared with unstressed controls, $P<0.05$ (B).

A

Collagen I

Stretch Stress   −   +

p-p38 →

pan p38 →

p53 →

B

Stretch stress (min)   0   5   10   30   60

p-p38 →

pan p38 →

C

GST-p53   MBP

Stretch stress (min)   −   +   −   +

D

ras N17   rac1 N17   neo

Stretch stress   −   +   −   +   −   +

p-p38 →

E

ras N1   rac1 N17   neo

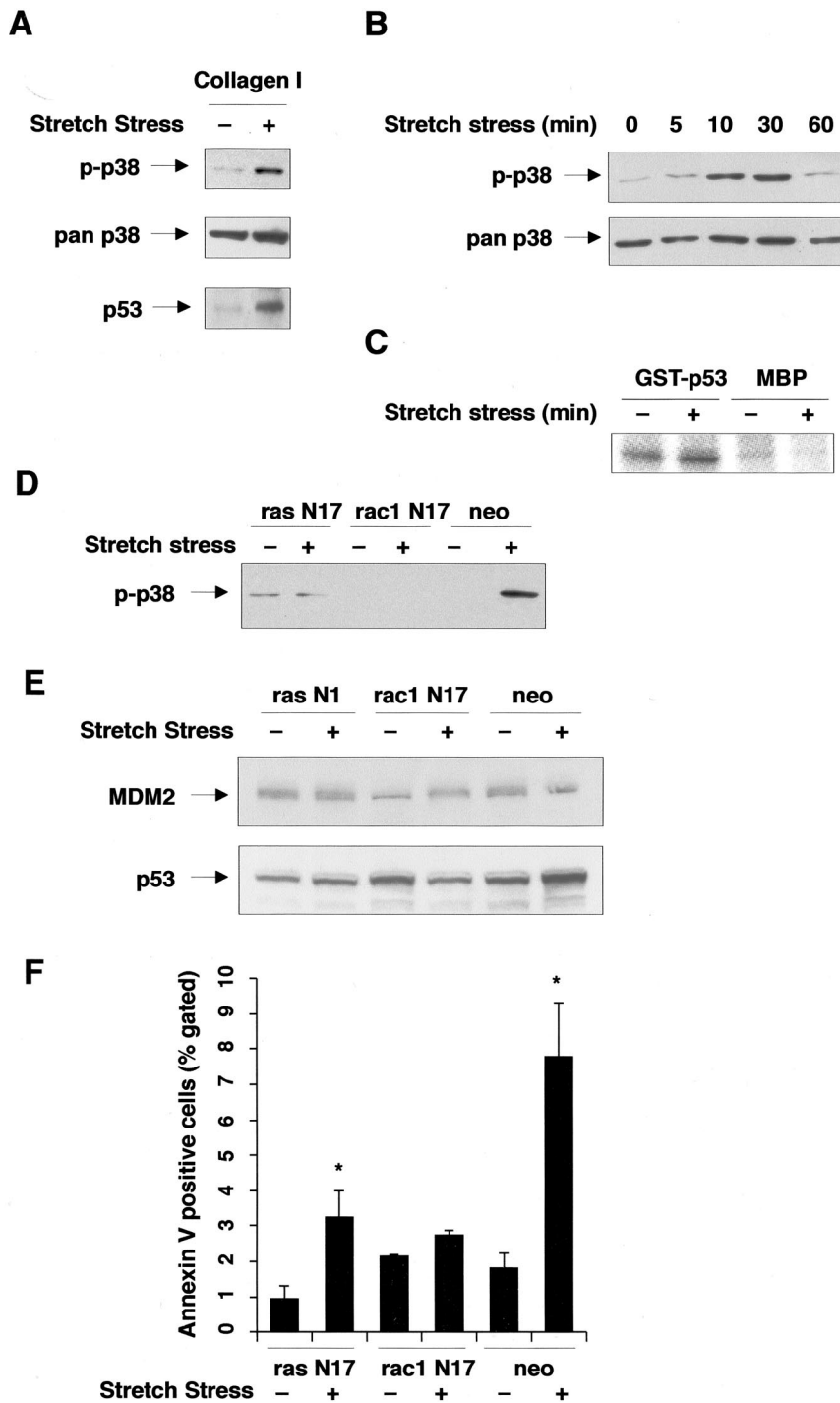Stretch Stress   −   +   −   +   −   +

MDM2 →

p53 →

F

Figure 2. Stretch-induced apoptosis signaling is matrix-dependent and involves activation of *Ras/Rac*, p38 MAPK, and p53. SMCs were cultivated on flexible silicone elastomer strain plates pre-coated with collagen I and subjected to cyclic mechanical stress for 6 hours (p53) or 15 minutes (phosphorylated (p-) p38). A and B, Results of Western blot analysis of p-p38 and p53. pan p38 shows total p38 proteins labeled with anti–pan p38 antibody. C, SMCs were serum-starved for 3 days and stretch-stressed for 15 minutes. Protein extracts and kinase activity was measured on the basis of phosphorylation of GST-p53 substrate. The specificity of the band was determined by using myelin basic protein as a substrate. D through F, Rat SMCs stably expressing dominant negative *Ras* (*Ras* N17) or dominant negative *Rac* (*Rac* N17) were cultivated and treated as described above. D and E, Results of Western blot analysis of p-p38 (D), MDM2 (E), and p53 (E). F, Apoptotic cells were assessed by annexin V labeling and FACS analysis after stretch treatment. Bar graph represents mean values ($\pm$SEM) of 3 independent experiments. *Significant difference compared with unstressed controls, $P<0.05$ (F).

stretch–induced phosphorylation of p38 (Figure 2D) and inhibited stretch-stimulated expression of p53 (Figure 2E). Interestingly, the expression profile of the negative p53-inhibitor, MDM2, was reciprocally proportional to the p53 expression (Figure 2E). The absence of functional *Ras/Rac* blocked stretch-induced apoptosis (Figure 2F). The increase in apoptotic cells in *Ras1* N17 SMCs was still significant but much lower than in the control cells containing the vector only (neo). Therefore, the small G proteins *Rac*, and, to a lower extent, *Ras*, appear to transduce proapoptotic signals in response to mechanical stress.

Expression of Integrin β_1 Increases Time-Dependently on Mechanical Stretch

The above findings on different ECMs suggest first, that different sets of receptors (mediating adhesion to different ECM proteins) mediate different mechanical stress–induced signaling pathways in SMCs, and, second, that receptors mediating the binding to collagen I, such as β_1-integrin–containing receptors, may be particularly involved in signaling leading to mechanical stress–induced SMC apoptosis. After 24 hours of cyclic stretch, we observed a significant increase in the expression of β_1-integrin (Figure 3A). In
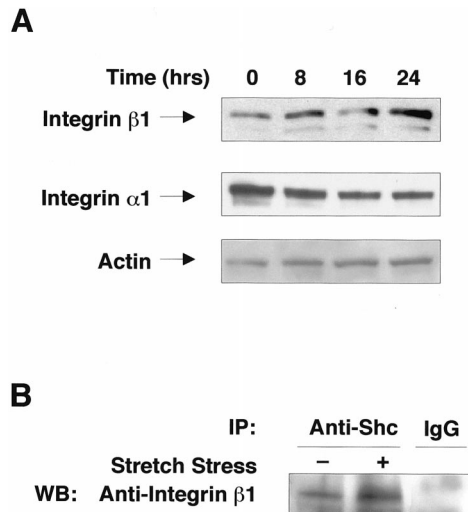
A

B

Figure 3. Mechanical stretch increases expression of integrin β_1 and integrin β_1-*Shc* interaction. For indicated times, SMCs were subjected to cyclic mechanical stress. A, Results of Western blot analysis of integrin β_1, integrin α_1, and actin. For detection of *Shc*-integrin β_1 immunocomplexes, SMCs were stretched for 24 hours and protein extracts were immunoprecipitated (IP) with an anti-*Shc* antibody, followed by Western blotting (WB) with anti–integrin β_1 (B). Control is normal mouse IgG (IgG). Data represent similar results from 3 independent experiments.

contrast, α_1-integrin did not increase in response to mechanical stress. Furthermore, β_1-integrin associated with the adapter protein *Shc* on mechanical stretch, indicating active integrin signaling (Figure 3B).

Cytochalasin B or Anti–Integrin β_1 But Not Suramin Can Inhibit the Activation of p38 MAPK and p53

To clarify whether integrin signaling is required for stretch-induced p38 MAPK phosphorylation and p53 expression after mechanical stretch, SMCs were pretreated with cytochalasin B (1 μmol/L) to disrupt the actin filaments and thus integrin signaling or preincubated with an anti–integrin-β_1–blocking antibody (10 μg/mL) (Figures 4A, 4D, and 4E). Surprisingly, cytochalasin B completely abrogated p38 MAPK phosphorylation (Figure 4A) and reduced the increase of p53 protein levels (Figure 4D) after stress. Specific blocking of integrin β_1–containing receptors with an anti–integrin-β_1–blocking antibody had similar effects and partly inhibited the mechanical stretch–induced increase in p53 protein levels (Figure 4E).

Previously, we demonstrated that mechanical stress stimulates PDGF receptor phosphorylation, which can be inhibited by suramin, a broad-spectrum growth factor receptor antagonist.[26] Similarly, treatment with suramin blocked phosphorylation of PDGF receptor-α and ERK1/2, commonly associated with signaling pathways leading to enhanced proliferation but not phosphorylation of p38 MAPK (Figure 4B). As expected, treatment with suramin had no influence on increased p53 expression after mechanical stress (Figure 4C). These data indicate that integrin signaling but not growth factor receptor–ERK pathways are involved in signal transduction leading to stretch-induced p53 expression.
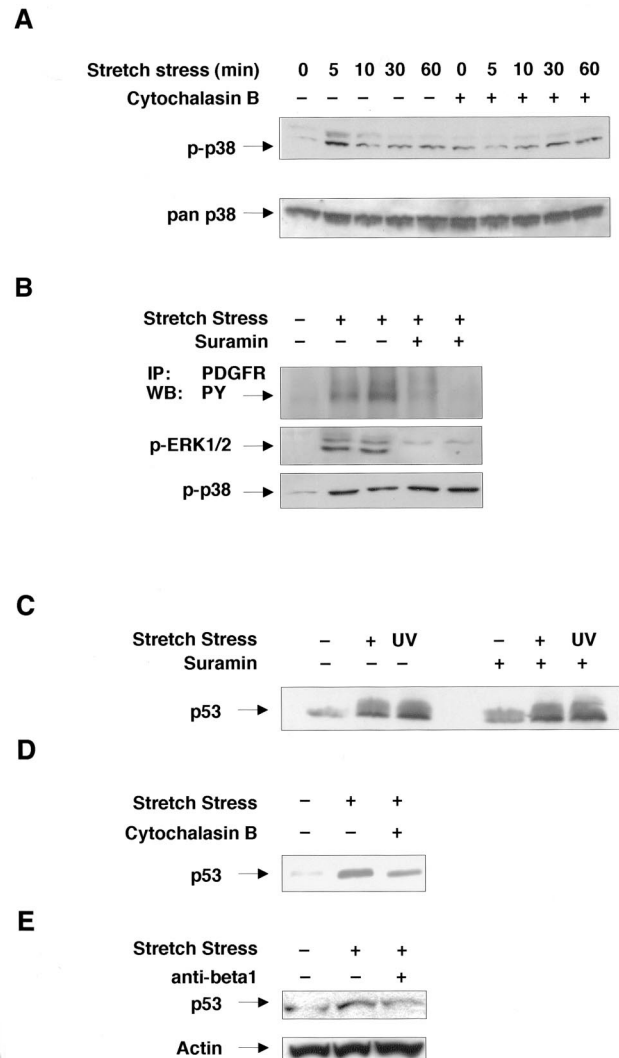
A

B

C

D

E

Figure 4. Cytochalasin B and anti–integrin β_1 but not suramin inhibits stretch-induced p38 MAPK phosphorylation and p53 expression. SMCs were treated with cytochalasin B (1 μmol/L) or anti–integrin β_1 (10 μg/mL) and subjected to cyclic mechanical stress. Results of Western blot analysis of phosphorylated (p) p38 (A) and p53 (D and E) are shown. pan p38 shows total p38 proteins labeled with anti–pan p38 antibody (A). For immunoprecipitation of PDGFR, serum-starved SMCs were incubated with suramin (0.3 mmol/L) for 30 minutes and subjected to cyclic mechanical stretch for 8 minutes. Total protein extracts were prepared and immunoprecipitated (IP) with a polyclonal antibody against PDGFRα followed by Western blotting (WB) with anti-phosphotyrosine (PY) (B). Two lower panels (B) show results of Western blot analysis for phosphorylated (p) ERK 1/2 and p38. For analysis of p53 expression, experimental procedures were similar to those described in D and E. Results of Western blot analysis of p53 levels are shown (C).

Cytochalasin B and β_1-Integrin Blocking But Not Suramin Inhibit Mechanical Stretch–Induced Apoptosis

To further elucidate the role of integrins, in particular the involvement of integrin β_1 in mechanical stretch–induced apoptosis, cells were pretreated with cytochalasin B (Figure 5A), with a β_1-integrin–blocking antibody (Figure 5B), or with suramin (Figure 5C) and subjected to cyclic mechanical stretch. Both cytochalasin B and anti–integrin β_1 but not
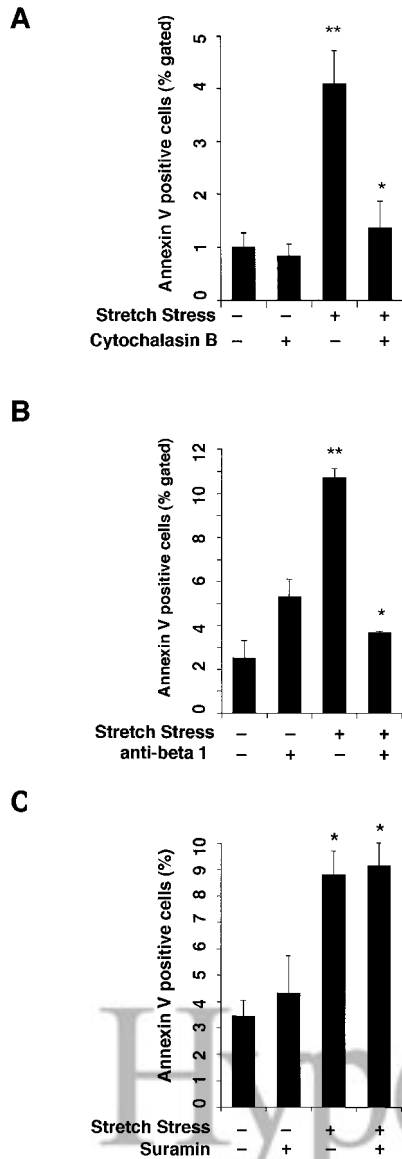
A

B

C

Figure 5. Inhibition of stretch-induced apoptosis by treatment with cytochalasin B and anti–integrin β_1 but not suramin. SMCs were treated with cytochalasin B (1 μmol/L) (A), with anti–integrin β_1 (10 μg/mL) (B), or with suramin (0.3 mmol/L) (C) for 1 hour and then subjected to cyclic mechanical stretch. Apoptotic cells were assessed by annexin V labeling and FACS analysis. Bar graphs represent mean values (±SEM) of 3 independent experiments. *Significant difference compared with unstressed controls. **Significant difference compared with positive controls, $P<0.05$.

suramin effectively inhibited stretch-induced apoptosis suggesting that integrins, integrin β_1 in particular, are at least in part responsible for mechanical stress–induced apoptosis in SMCs.

Involvement of the PKC Isoform δ in Mechanical Stretch–Induced Apoptosis

There are various indications linking PKC isoforms directly or indirectly to integrin complexes[30] and that mechanical stretch activates PKCδ in SMCs.[31,32] In this study, we show
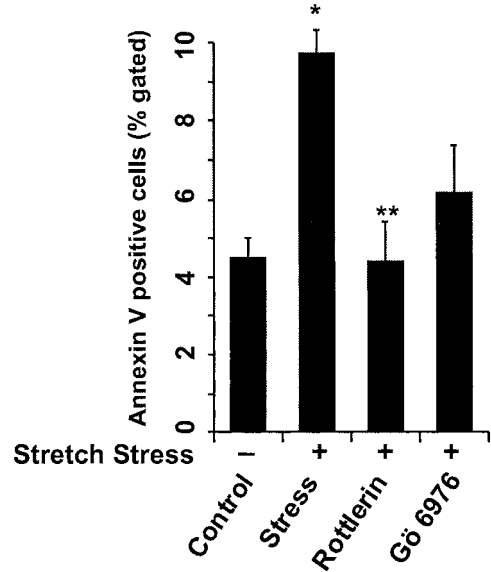

Figure 6. PKCδ is involved in integrin-mediated apoptosis induced by mechanical stress in SMCs. SMCs were treated with Rottlerin (10 μmol/L) or Gö 6976 (10 nmol/L) for 1 hour and then subjected to cyclic mechanical stretch. Apoptotic cells were assessed by annexin V labeling and FACS analysis. Bar graphs represent mean values (±SEM) of 3 independent experiments. *Significant difference compared with unstressed controls, $P<0.05$. **Significant difference compared stressed SMCs without PKC inhibitor, $P<0.05$.

that functional inhibition of PKCδ by the specific inhibitor Rottlerin abrogated stretch-induced apoptosis in SMCs. Inhibition of PKCα by the specific inhibitor Gö 6976 could not significantly reduce stretch-induced apoptosis in SMCs (Figure 6).

Discussion

In vessels, a certain level of mechanical stress in the form of hemodynamic force is essential to develop and maintain a differentiated and functional SMC phenotype.[33] Cultured SMCs increase SMC-specific markers when they are subjected to cyclic mechanical stretch.[34] In cardiomyocytes, overstretching induces signaling pathways that induce growth inhibition and apoptosis.[35,36] Similar findings have been described for SMCs by us and others.[11,37] In recent years, apoptosis of SMCs has been implicated in both development and outcome of atherosclerosis.[7–10,38] The altered balance between apoptosis and proliferation appears to promote disease development. An initial increase of apoptosis might trigger SMC proliferation, whereas an overall decrease in apoptosis, as it was observed in $p53^{-/-}$[12] and $PKC\delta^{-/-}$ mice,[13] leads to increased atherosclerotic lesions. In the current report, we provide the first evidence that integrins, β_1-integrin–containing receptors in particular, are involved in mechanical stretch–induced apoptosis.

Integrins not only mediate adhesion but function also as "proper" signaling receptors. Integrin signaling is commonly associated with cell survival.[39] Loss of adhesion leads to cell death, a phenomenon termed anoikosis (homelessness).[40] We showed that mechanical stretch induces apoptosis in SMCs
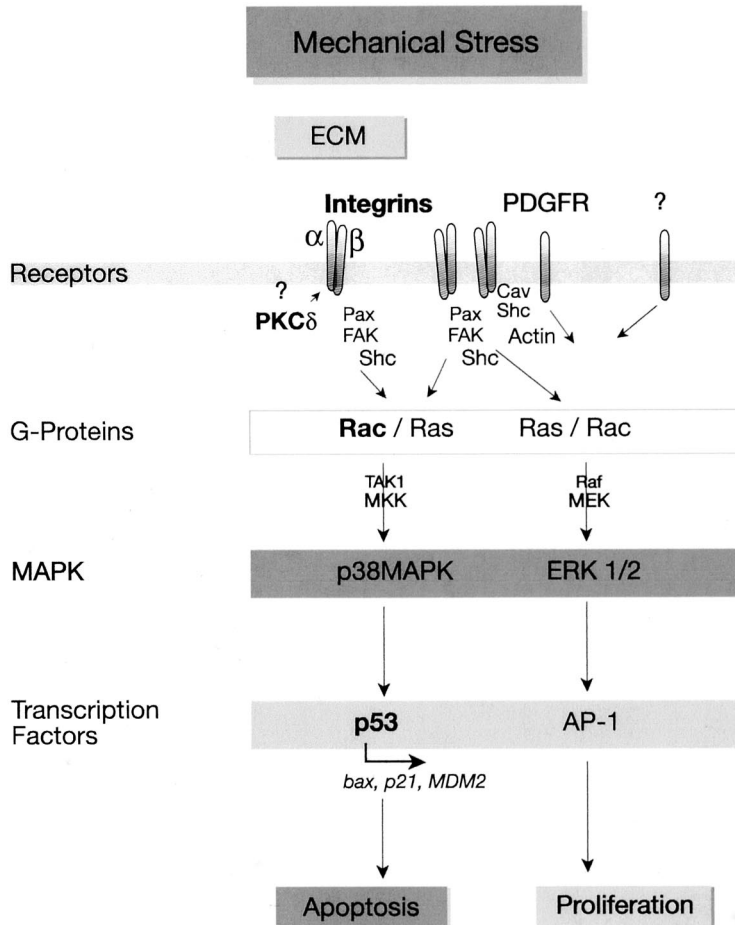
Figure 7. Schematic model of mechanical stress signaling in SMCs. Various surface receptors, including integrins and growth factor receptors, transduce mechanical stress exerted by hemodynamic forces. Integrins mediate both adhesion to ECM and signals resulting from mechanical forces leading to their activation and binding of various adapter proteins, such as Paxillin (Pax), focal adhesion kinase (FAK), and *Shc,* and activation of *Ras* and *Rac*. PKCδ appears to have an important function in regulating the cell cycle and apoptosis. Depending on the signal, activation of different sets of MAPKs leads to different cellular responses. Mechanical stress–stimulated integrin β_1 and p38 MAPK activate p53, ultimately leading to growth arrest and apoptosis. Both apoptosis and proliferation pathways share many common elements, and they converge and influence each other at different levels.

cultured on a collagen I matrix. This finding is particularly interesting in the light of the pathogenesis of atherosclerosis. Injured SMCs increase the production of collagen, particularly collagen type I.[41] Atherosclerosis is associated with elevated mechanical stretch leading to SMC proliferation, migration, and abnormal ECM accumulation.[6] The ECM is produced, at least in part, by SMCs themselves, reflecting an adaptive change to altered conditions. Increased collagen deposition can be found in advanced intimal lesions, particularly in coronary artery plaques.[41] Mechanical strain induces ECM protein synthesis (fibronectin and collagen) and the activity of matrix-degrading enzymes and matrix metalloproteinase (MMP-2) in cultured SMCs, which reflects a change in ECM composition.[42] Conversely, ECM profoundly influences and modulates the cell cycle of SMCs in response to mechanical stress.[41,43,44]

Our experimental results showed that receptors linked to collagen I binding mediate stretch-induced apoptosis in cultured SMCs. The fact that a β_1-integrin–blocking antibody inhibited stretch-induced apoptosis strengthened this hypothesis and suggests that β_1-integrin–containing receptors are particularly involved (Figure 5B). We further observed that mechanical stretch–induced integrin β_1 protein expression and binding of the adapter protein *Shc* to integrin β_1 (Figures 3A and 3B). This is consistent with the findings of others who showed that shear stress induced an association of β_1-integrin with *Shc* in endothelial cells.[21] No prediction in terms of the

functional outcome, that is, proliferation or cell death, can be made from integrin β_1 protein expression, *Shc* binding to integrins, or, the activation of the small GTP-binding proteins *Ras* and *Rac*, which have been shown to mediate both mechanical stress–induced proliferation and apoptosis.[32] The dissection of the 2 pathways appears to occur further downstream, at the level of MAPK, and presumably depends on the initial signal as well as on other converging signals, such as those resulting from growth factor receptors.[15] Both growth factor receptors and integrin receptors share elements of their signaling pathways and may influence each other at different levels. In the current report, we could clearly show that the stretch-apoptosis signaling pathway is mediated by *Rac*-p38 MAPK, which stimulates p53. p53 is an essential element of this pathway, since lack of p53 prevented stretch-induced apoptosis.[12] The fact that both apoptosis and expression of 53 depend on ECM (the involvement of a different set of integrins) and that it can be blocked by cytochalasin B treatment and an integrin-β_1–blocking antibody but not suramin strongly suggests that integrins represent important mechanosensors. By contrast, suramin blocks growth factor receptor–ERK pathways and proliferation of SMCs.

We have shown previously that PKCδ$^{-/-}$ SMCs were resistant to apoptosis compared with wild-type SMCs.[13] The results that specific functional blocking of PKCδ inhibits stretch-induced apoptosis are consistent with our previous observations made in mice.[13] This underlines the important

role of members of the PKC family, PKCδ in particular, in signaling pathways leading to apoptosis in response to mechanical stress. We therefore propose the following model of mechanical stress signaling in SMC, as shown in Figure 7: Mechanical stress exerted by hemodynamic forces is sensed by various surface receptors, including integrins and growth factor receptors. Integrins mediate adhesion but also signals resulting from the ECM and mechanical force leading to conformational change and activation, binding of various adaptor proteins, such as *Shc,* and activation of *Ras* and *Rac,* which in turn can initiate MAPK cascades and ultimately influence the cell cycle. PKCδ also appears to play a crucial role in controlling the cell cycle. Depending on the kind of signal, diverse sets of integrins and MAPKs are activated. Activation of integrin β_1 and p38 MAPK triggers activation of p53, resulting in apoptotic cell death.

Perspectives

The complex interplay in signaling leading to either cell growth or apoptosis decides a cell's destiny. Apoptosis of SMCs often reflects an immediate response to altered external conditions, such as increased mechanical stress in hypertension. This response can be beneficial because it provides the opportunity for tissue remodeling and adaptation. If the stress persists and becomes overwhelmingly increased, apoptosis and proliferation may lead to disturbed remodeling and finally pathological conditions. Further studies focusing on how SMCs effectively switch from one signal to another leading to growth or apoptosis should provide valuable information for the design of new drugs for therapeutic intervention in cardiovascular diseases.

References

1. Lehoux S, Tedgui A. Signal transduction of mechanical stresses in the vascular wall. *Hypertension*. 1998;32:338–345.
2. Henry JP, Grim CE. Psychosocial mechanisms of primary hypertension. *J Hypertens*. 1990;8:783–793.
3. Dzau VJ, Gibbons GH, Morishita R, Pratt RE. New perspectives in hypertension research: potentials of vascular biology. *Hypertension*. 1994;23:1132–1140.
4. Hollander W. Role of hypertension in atherosclerosis and cardiovascular disease. *Am J Cardiol*. 1976;38:786–800.
5. Kleinstreuer C, Hyun S, Buchanan JR Jr, Longest PW, Archie JP Jr, Truskey GA. Hemodynamic parameters and early intimal thickening in branching blood vessels. *Crit Rev Biomed Eng*. 2001;29:1–64.
6. Xu Q. Biomechanical-stress-induced signaling and gene expression in the development of arteriosclerosis. *Trends Cardiovasc Med*. 2000;10:35–41.
7. Geng YJ, Libby P. Evidence for apoptosis in advanced human atheroma: colocalization with interleukin-1 beta-converting enzyme. *Am J Pathol*. 1995;147:251–266.
8. Isner JM, Kearney M, Bortman S, Passeri J. Apoptosis in human atherosclerosis and restenosis. *Circulation*. 1995;91:2703–2711.
9. Newby AC, George SJ. Proliferation, migration, matrix turnover, and death of smooth muscle cells in native coronary and vein graft atherosclerosis. *Curr Opin Cardiol*. 1996;11:574–582.
10. Kockx MM. Apoptosis in the atherosclerotic plaque: quantitative and qualitative aspects. *Arterioscler Thromb Vasc Biol*. 1998;18:1519–1522.
11. Mayr M, Li C, Zou Y, Huemer U, Hu Y, Xu Q. Biomechanical stress-induced apoptosis in vein grafts involves p38 mitogen-activated protein kinases. *FASEB J*. 2000;14:261–270.
12. Mayr U, Mayr M, Li C, Wernig F, Dietrich H, Hu Y, Xu Q. Loss of p53 accelerates neointimal lesions of vein bypass grafts in mice. *Circ Res*. 2002;90:197–204.
13. Leitges M, Mayr M, Braun U, Mayr U, Li C, Pfister G, Ghaffari-Tabrizi N, Baier G, Hu Y, Xu Q. Exacerbated vein graft arteriosclerosis in protein kinase Cdelta-null mice. *J Clin Invest*. 2001;108:1505–1512.
14. Mayr M, Xu Q. Smooth muscle cell apoptosis in arteriosclerosis. *Exp Gerontol*. 2001;36:969–987.
15. Wernig F, Xu Q. Mechanical stress-induced apoptosis in the cardiovascular system. *Prog Biophys Mol Biol*. 2002;78:105–137.
16. Eliceiri BP. Integrin and growth factor receptor crosstalk. *Circ Res*. 2001;89:1104–1110.
17. Intengan HD, Schiffrin EL. Structure and mechanical properties of resistance arteries in hypertension: role of adhesion molecules and extracellular matrix determinants. *Hypertension*. 2000;36:312–318.
18. Wilson E, Sudhir K, Ives HE. Mechanical strain of rat vascular smooth muscle cells is sensed by specific extracellular matrix/integrin interactions. *J Clin Invest*. 1995;96:2364–2372.
19. Goldschmidt ME, McLeod KJ, Taylor WR. Integrin-mediated mechanotransduction in vascular smooth muscle cells: frequency and force response characteristics. *Circ Res*. 2001;88:674–680.
20. Li S, Kim M, Hu YL, Jalali S, Schlaepfer DD, Hunter T, Chien S, Shyy JY. Fluid shear stress activation of focal adhesion kinase: linking to mitogen-activated protein kinases. *J Biol Chem*. 1997;272:30455–30462.
21. Chen KD, Li YS, Kim M, Li S, Yuan S, Chien S, Shyy JY. Mechanotransduction in response to shear stress: roles of receptor tyrosine kinases, integrins, and Shc. *J Biol Chem*. 1999;274:18393–18400.
22. Xu Q, Li DG, Holbrook NJ, Udelsman R. Acute hypertension induces heat-shock protein 70 gene expression in rat aorta. *Circulation*. 1995;92:1223–1229.
23. Hu Y, Zou Y, Dietrich H, Wick G, Xu Q. Inhibition of neointima hyperplasia of mouse vein grafts by locally applied suramin. *Circulation*. 1999;100:861–868.
24. Li C, Hu Y, Mayr M, Xu Q. Cyclic strain stress-induced mitogen-activated protein kinase (MAPK) phosphatase 1 expression in vascular smooth muscle cells is regulated by Ras/Rac-MAPK pathways. *J Biol Chem*. 1999;274:25273–25280.
25. Dietrich H, Hu Y, Zou Y, Huemer U, Metzler B, Li C, Mayr M, Xu Q. Rapid development of vein graft atheroma in ApoE-deficient mice. *Am J Pathol*. 2000;157:659–669.
26. Hu Y, Bock G, Wick G, Xu Q. Activation of PDGF receptor alpha in vascular smooth muscle cells by mechanical stress. *FASEB J*. 1998;12:1135–1142.
27. Hu Y, Metzler B, Xu Q. Discordant activation of stress-activated protein kinases or c-Jun NH2- terminal protein kinases in tissues of heat-stressed mice. *J Biol Chem*. 1997;272:9113–9119.
28. Metzler B, Hu Y, Sturm G, Wick G, Xu Q. Induction of mitogen-activated protein kinase phosphatase-1 by arachidonic acid in vascular smooth muscle cells. *J Biol Chem*. 1998;273:33320–33326.
29. Li C, Hu Y, Sturm G, Wick G, Xu Q. Ras/Rac-Dependent activation of p38 mitogen-activated protein kinases in smooth muscle cells stimulated by cyclic strain stress. *Arterioscler Thromb Vasc Biol*. 2000;20:E1–E9.
30. Giancotti FG. Complexity and specificity of integrin signalling [news]. *Nat Cell Biol*. 2000;2:E13–E14.
31. Kuchan MJ, Frangos JA. Shear stress regulates endothelin-1 release via protein kinase C and cGMP in cultured endothelial cells. *Am J Physiol*. 1993;264:H150–H156.
32. Li C, Xu Q. Mechanical stress-initiated signal transductions in vascular smooth muscle cells. *Cell Signal*. 2000;12:435–445.
33. Birukov KG, Bardy N, Lehoux S, Merval R, Shirinsky VP, Tedgui A. Intraluminal pressure is essential for the maintenance of smooth muscle caldesmon and filamin content in aortic organ culture. *Arterioscler Thromb Vasc Biol*. 1998;18:922–927.
34. Reusch P, Wagdy H, Reusch R, Wilson E, Ives HE. Mechanical strain increases smooth muscle and decreases nonmuscle myosin expression in rat vascular smooth muscle cells. *Circ Res*. 1996;79:1046–1053.

35. Leri A, Claudio PP, Li Q, Wang X, Reiss K, Wang S, Malhotra A, Kajstura J, Anversa P. Stretch-mediated release of angiotensin II induces myocyte apoptosis by activating p53 that enhances the local renin-angiotensin system and decreases the Bcl-2-to-Bax protein ratio in the cell. *J Clin Invest*. 1998;101:1326–1342.

36. Cheng W, Li B, Kajstura J, Li P, Wolin MS, Sonnenblick EH, Hintze TH, Olivetti G, Anversa P. Stretch-induced programmed myocyte cell death. *J Clin Invest*. 1995;96:2247–2259.

37. Sotoudeh M, Li YS, Yajima N, Chang CC, Tsou TC, Wang Y, Usami S, Ratcliffe A, Chien S, Shyy JY. Induction of apoptosis in vascular smooth muscle cells by mechanical stretch. *Am J Physiol Heart Circ Physiol*. 2002;282:H1709–H1716.

38. Han DK, Haudenschild CC, Hong MK, Tinkle BT, Leon MB, Liau G. Evidence for apoptosis in human atherogenesis and in a rat vascular injury model. *Am J Pathol*. 1995;147:267–277.

39. Bombeli T, Schwartz BR, Harlan JM. Endothelial cells undergoing apoptosis become proadhesive for nonactivated platelets. *Blood*. 1999;93:3831–3838.

40. Frisch SM, Ruoslahti E. Integrins and anoikis. *Curr Opin Cell Biol*. 1997;9:701–706.

41. Assoian RK, Marcantonio EE. The extracellular matrix as a cell cycle control element in atherosclerosis and restenosis. *J Clin Invest*. 1996;98:2436–2439.

42. O'Callaghan CJ, Williams B. Mechanical strain-induced extracellular matrix production by human vascular smooth muscle cells: role of TGF-beta(1). *Hypertension*. 2000;36:319–324.

43. Newby AC, Zaltsman AB. Fibrous cap formation or destruction: the critical importance of vascular smooth muscle cell proliferation, migration and matrix formation. *Cardiovasc Res*. 1999;41:345–360.

44. Koyama H, Raines EW, Bornfeldt KE, Roberts JM, Ross R. Fibrillar collagen inhibits arterial smooth muscle proliferation through regulation of Cdk2 inhibitors. *Cell*. 1996;87:1069–1078.

American Heart Association®

Fighting Heart Disease and Stroke

Hypertension

JOURNAL OF THE AMERICAN HEART ASSOCIATION