Apoptosis of smooth muscle cells (SMC) in atherosclerotic vessels can destabilize the atheroma plaque and result in rupture, thrombosis, and sudden death. In efforts to understand the molecular processes regulating apoptosis in this cell type, we have defined a novel mechanism involving the ubiquitously expressed transcription factor Sp1. Subtypes of SMC expressing abundant levels of Sp1 produce the death agonist, Fas ligand (FasL) and undergo greater spontaneous apoptosis. Sp1 activates the FasL promoter via a distinct nucleotide recognition element whose integrity is crucial for inducible expression. Inducible FasL promoter activation is also inhibited by a dominant-negative form of Sp1. Increased SMC apoptosis is preceded by Sp1 phosphorylation, increased FasL transcription, and the autocrine/paracrine engagement of FasL with its cell-surface receptor, Fas. Inducible FasL transcription and apoptosis are blocked by dominant-negative protein kinase C-ζ, whose wild-type counterpart phosphorylates Sp1. Thus, Sp1 phosphorylation is a proapoptotic transcriptional event in vascular SMC and, given the wide distribution of this housekeeping transcription factor, may be a common regulatory theme in apoptotic signal transduction.

Apoptosis is a genetically regulated “programmed” form of cell death and is characterized by a number of specific biochemical and morphological changes, including nuclear chromatin condensation, cytoplasmic condensation, membrane blebbing, and internucleosomal fragmentation of DNA (1, 2). Fas/APO-1 (or CD95) is a 45-kDa cell surface glycoprotein that belongs to the tumor necrosis factor receptor superfamily and mediates apoptosis in various normal and transformed cell types. Upon the engagement of Fas by Fas ligand (FasL), a highly conserved, ubiquitously expressed 40-kDa glycoprotein, the apoptotic cysteine protease caspase-8/FLICE is recruited to the receptor via FADD and activated by proteolysis (3). Caspase-3/CPP32, which is expressed in cells as the inactive 32-kDa form is, in turn, cleaved by caspase-8/FLICE to produce two mature subunits (17 and 12 kDa). Active caspase-3/CPP32 cleaves nuclear mitotic apparatus protein and mediates DNA fragmentation, chromatin condensation, and the formation of apoptotic bodies (4).

FasL (5) and Fas (6, 7) are both expressed in arterial tissue, including the human atherosclerotic plaque. Immunohistochemical analysis revealed FasL expression in 34 of 34 carotid atherosclerotic plaques examined, with virtually all FasL positive-staining associated with intimal smooth muscle cells (SMCs) and little staining apparent in normal arterial tissue (5). Fas is also highly expressed in intimal SMCs of the plaque (6, 7). FasL/Fas expression and apoptosis (8–10) in normal artery and plaque has prompted speculation on the roles of these molecular mediators in vascular cells. Apoptosis in diseased tissue may inhibit arterial thickening by limiting cell proliferation and accumulation in the intima (6). In atherosclerotic tissue, apoptosis particularly of collagen-producing SMCs may substantially weaken the plaque causing it to rupture, initiate thrombosis, and trigger acute coronary syndromes (11–12). Overexpression of FasL in balloon-injured rat carotid arteries devoid of endothelium-induced apoptosis in medial SMCs and inhibited intimal hyperplasia (13, 14). However, recent evidence in a rabbit model suggests that FasL may promote rather than retard atherogenesis. FasL overexpression in nondenuded arteries of hypercholesterolemic animals stimulated lesion formation in these animals via increased cellularity (15). These observations may be due to differences in artery and lesion cellular composition or cholesterol feeding between the two animal models.

Despite clear evidence for FasL and Fas expression in SMCs of the artery wall, the molecular mechanisms mediating FasL production in vascular cells are presently not known. The promoter region of the FasL gene has recently been cloned and found to contain binding sites for a number of transcription factors including NF-κB (16), AP-1 (16), NFAT (17), ATF2 (18), Egr-2 (17), and Egr-3 (17). The promoter contains a single regulatory region within a 2.3-kilobase portion of the 5′-untranslated genome (19). Analysis of the FasL promoter has mostly been confined to T cells. For example, T cell activation following CD4 cross-linking induces NFAT binding to the FasL enhancer and gene transactivation (19). Similarly, cytotoxic stress-induced FasL expression involves the activation of NF-κB, AP-1, and c-Jun N-terminal kinase, prior to cell death (16–20). Activity of the FasL promoter is also regulated by MEK kinase-1 (18). However, transcription factor phosphorylation has not yet been directly demonstrated as a prerequisite step in apoptosis.

The discovery and functional characterization of Sp1 as a GC-rich binding nuclear protein has provided a useful paradigm to our understanding of the regulation of transcriptional...
activation in eukaryotic cells (21, 22). Sp1 is a broadly expressed nuclear protein of ~100 kDa and contains three Kruppel-like zinc fingers that contact DNA (21, 22). A nucleotide recognition element for Sp1 is located in the FasL promoter at position −281/−276 base pairs (GGGGCG) relative to the transcriptional start site. Sp1 can influence gene expression as changes in its nuclear concentration and interaction with the promoter, by providing architectural support, serving as a co-factor, or by undergoing chemical modification. Sp1 phosphorylation e.g. mediates inducible tissue factor expression in vascular endothelial cells exposed to fluid shear stress (23). The significance of Sp1 in the process of apoptosis in any cell type is presently unknown. This knowledge would advance our understanding of the transcriptional basis of extrinsic apoptosis, given the wide distribution of both Sp1 and FasL.

WKY12-22 and WKY3M-22 cells are well established subtypes of vascular smooth muscle cells that are phenotypically distinct (24, 25). WKY12-22 cells have a cobblestone morphology in culture, proliferate in plasma-derived serum (which lacks vital growth factors), and spontaneously overexpress mRNA for platelet-derived growth factor (PDGF) B-chain, elastin, and osteopontin (24, 25). In contrast, WKY3M-22 cells are typically spindle-shaped and do not express PDGF-B, elastin, or osteopontin mRNA, nor do they grow in plasma-derived serum. Both cell subtypes are phenotypically stable in culture and can be passaged indefinitely. Therefore, WKY12-22 and WKY3M22 cells represent important cells with which to delineate the molecular basis for differences in SMC phenotype and gene expression.

We recently reported that Sp1 is spontaneously expressed at higher levels in WKY12-22 cells than WKY3M-22 cells and that as a consequence, Sp1-dependent genes, such as PDGF-B, are overexpressed in WKY12-22 cells compared with its sister cell subtype (26). These observations provided important insight into the transcriptional basis for differential gene expression. Here we explored the regulatory role of Sp1 in inducible FasL expression and apoptosis in two phenotypically distinct SMC subtypes.

**Experimental Procedures**

**Transfections and Luciferase Assays—**SMCs were maintained in Waymouth’s medium (Life Technologies, Inc.), pH 7.4, containing 10% fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO2. Transient transfections were performed with cells at 60% confluence, and the indicated constructs together with 2 μg of the internal control vector, pRL-TK, using FuGENE6 transfection agent (Roche Molecular Biochemicals). After 24 h, the transfected cells were incubated with or without 5 units of CIP (final concentration determined by titration experiments with 3P-labeled FasL Oligo) at 37 °C for 5 min and then on ice for 15 min. The reaction was quenched by the addition of solution A (10 mM Hepes, pH 7.9, 1.5 mM MgCl2, 10 mM KCl) and then on ice for 15 min. Four hundred microliters of binding buffer was added to each tube, and annexin V staining was analyzed by flow cytometry within 1 h. Results are expressed as annexin V staining as a percentage of the total cell population.

**Propidium Iodide Nuclear Staining—**SMCs were grown in chamber slides (80% confluent) and incubated with CAM (1 μg/ml) for 24 h. The cells were washed in PBS, pH 7.4, and fixed with methanol/acetic acid (50:50) for 10 min at 22 °C. Propidium iodide (50 μl) was added to each well and incubated for a maximum of 5 min followed by a second wash with PBS. Cells undergoing apoptosis were visualized by confocal microscopy.

**Nuclear Extract Preparation—**SMCs treated with CAM for various times were washed and scraped in 10 ml of PBS and transferred to 50-ml centrifuge tubes. Samples were spun at 14,000 rpm for 5 min at 4 °C. The pellet was resuspended in 100 μl (for two-100-mm-dishes) of solution A (10 mM Hepes, pH 7.9, 1.5 mM MgCl2, 10 mM KCl) and then on ice for 5 min. Samples were spun at 14,000 rpm for 40 s. The pellet was resuspended in 20 μl of solution C (20 mM Hepes, pH 7.9, 1.5 mM MgCl2, 420 mM NaCl, 0.2 mM EDTA) and mixed gently for 20 min at 4 °C. The supernatant was transferred to precooled Eppendorf tubes containing 20 μl of solution D (20 mM Hepes, pH 7.9, 1.5 mM KCl, 0.2 mM EDTA, 20% glycerol) and stored at −80 °C until use. All buffers contained protease inhibitors.

**Electrophoretic Mobility Shift Assay (EMSA)—**Nuclear extracts (6–10 μg) were incubated with 32P-labeled double-stranded oligonucleotide (150,000 cpm, 40 fmol) in 20 μl containing 10 mM Tris-HCl, pH 8.0, 50 mM MgCl2, 1 mM EDTA, 1 mM dithiothreitol, 5% glycerol, 1 μg of salmon sperm DNA, 5% sucrose, 1 μg of poly(dI-dC) and 1 μM poly[d(I-C)] and stored at −80 °C.

**RT-PCR—**Five micrograms of total RNA isolated using TRIzol reagent (Life Technologies, Inc.) were treated with DNase I, and cDNA was generated using Superscript II reverse transcriptase (Life Technologies, Inc.) according to random primers, and cDNA was generated using Transcriptor II cDNA synthesis kit (Roche Molecular Biochemicals). This assay, which measures cytoplasmic histone-associated internucleosomal DNA fragmentation, has been used previously to quantify inducible apoptosis in cultured cells (27–29). Briefly, the cells were washed gently in PBS and incubated with shaking in lysis buffer for 30 min at 22 °C. Lysates were transferred into Eppendorf tubes and spun at 14,000 rpm for 30 s. Twenty μl of the supernatant was used in the ELISA, which was performed in accordance with the manufacturer’s instructions and normalized to total cell number measured using a Coulter counter. Results are expressed as total internucleosomal DNA fragmentation as a proportion of the cell population.

**Quantitative Assessment of DNA Fragmentation—**Nuclear extracts were incubated with ice-cold phosphate-buffered saline, pH 7.4 and resuspended in 1× binding buffer (10 mM HEPES, pH 7.4, 140 mM NaCl, 2.5 mM CaCl2) at a concentration of 1 × 106 cells/ml. One hundred microliters of the suspension was transferred to 5-ml flat bottomed tubes where 5 μl of annexin V-fluorescein isothiocyanate and 10 μl of propidium iodide (50 μg/ml stock in PBS) was added. The cells were gently vortexed and incubated in the dark at 22 °C for 15 min. Four hundred microliters of binding buffer was added to each tube, and annexin V staining was analyzed by flow cytometry within 1 h. Results are expressed as annexin V staining as a percentage of the total cell population.

**Western Blot for Sp1—**Nuclear extracts were resolved by 8% SDS-polyacrylamide gel electrophoresis and binding complexes were visualized by autoradiography at −80 °C.

**Western Blotting and Immunoprecipitation—**Sp1 was detected with Sp1 polyclonal antibodies (1:1000, Santa Cruz Biotechnology) and subsequent chemiluminescent visualization.

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FIG. 1. Differences in spontaneous apoptosis and FasL expression in WKY12-22 and WKY3M-22 cells. A, spontaneous levels of apoptosis as determined by internucleosomal DNA fragmentation (left) and annexin V/FACS analysis (right) in WKY12-22 and WKY3M-22 cells. The y axis in the left and right panels represents total internucleosomal DNA fragmentation (as a proportion of the cell population) and annexin V staining (as a percentage of the total number of cells in the population), respectively. B, overexpression of Sp1 in WKY3M22 cells stimulates apoptosis. SMCs were transfected with 0, 1, 3, and 5 μg of CMV-Sp1 using FuGENE6 prior to quantification of apoptosis after 24 h by ELISA. Where required, the total amount of DNA transfected was supplemented to 5 μg with pcDNA3. C, dominant-negative Sp1 blocks apoptosis in WKY12-22 cells. SMCs were transfected with 40 μg of pEBGNLS or pEBGNLS-Sp1 using FuGENE6 prior to quantification of apoptosis after 24 h by ELISA. Results in B and C (ordinate axes) are expressed as total internucleosomal DNA fragmentation as a proportion of the total number of cells in the treatment population. D, WKY12-22 cells, but not WKY3M-22 cells, express FasL mRNA, and this is stimulated by CAM. Total RNA prepared from WKY12-22 cells incubated with CAM (1 μg/ml) for 24 h prior to reverse-transcription and PCR with the indicated primers. PCR cycle number is indicated in the figure. The densitometric assessment of the amplicons is represented histodiagramatically. E, comparative FasL mRNA expression in WKY12-22 cells and WKY3M-22 cells. Northern blot analysis was performed with total RNA of WKY3M-22 cells or WKY12-22 cells (with or without 24-h incubation with 1 μg/ml CAM. The data are representative of two independent determinations. Error bars represent S.E.
FIG. 2. Sp1 activates the FasL promoter in SMCs. Transient cotransfection analysis in WKY12-22 cells overexpressing Sp1 with FasL-hsLuc (A) and derivatives of FasL-hsLuc bearing 5′ deletions in the FasL promoter (B). 6 μg of pcDNA3 or CMV-Sp1 was used in B; 15 μg of FasL promoter-reporter construct was used throughout. Firefly luciferase activity was normalized to Renilla activity and the results were plotted as -fold increase relative to -296FasL-hsLuc or -271FasL-hsLuc, respectively. C, EMSA using [32P]FasL Oligo, [32P]mFasL Oligo, and nuclear extracts of WKY12-22 cells. EMSA was performed as described under “Experimental Procedures,” and nucleoprotein complexes were visualized by autoradiography. Arrows indicate nucleoprotein complexes, S denotes a supershift. Sequence of [32P]mFasL Oligo (−296/−265) is 5′-ATCAGAAAATTGTGGGCGGAAACTTCCAGG-3′, and [32P]mFasL Oligo is 5′-ATCAGAAAATTGTGGGCGGAAACTTCCAGG-3′; the mutation is underlined). D, mutation of the Sp1 site in FasL-hsLuc abrogates activation of the FasL promoter. E, Sp1 activation of FasL promoter in WKY3M-22 cells. 3 or 6 μg of pcDNA3 or CMV-Sp1 were used in cotransfection experiments with 15 μg of FasL promoter-reporter construct throughout. Firefly luciferase activity was normalized to Renilla activity, and the results were plotted as -fold increase relative to the pcDNA3 control in the FasL-hsLuc and mSp1FasL-hsLuc contransfectant groups, respectively. Error bars represent S.E. The data are representative of two independent determinations.
MgCl2, 2 mM dNTPs, 2.5 units of Taq DNA polymerase (Sigma), 5 μl of cDNA, and either 100 pmol of Fas primers, 100 pmol of FasL primers, or 20 pmol of β-actin primers using a Perkin Elmer thermocycler. Amplification was performed by denaturing the sample at 94 °C for 2 min, then cycling at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and an extension at 72 °C for 4 min. The number of PCR cycles for Fas/β-actin was 25. Twenty microliters of the reaction was visualized on 1.5% agarose gels with ethidium bromide staining.

Northern Blot Analysis—Fifteen μg of total RNA isolated using TRIzol reagent (Life Technologies, Inc.) was loaded onto a 1% formaldehyde/agarose gel and resolved by electrophoresis. Northern blot was performed as previously described (32). FasL and glyceraldehyde-3-phosphate dehydrogenase cDNA amplified by PCR were used as probes. The cDNA was labeled by nick translation (Roche Molecular Biochemicals).

RESULTS AND DISCUSSION

Sp1 Is Proapoptotic—To begin investigating a possible mechanistic role for Sp1 in programmed cell death, we compared apoptosis in two well established SMC subtypes isolated originally from the arteries of pup (2-week-old) and adult (3-month-old) rats (24, 25). Pup SMCs (WKY12-22 cells) are phenotypically distinct from their adult counterparts (WKY3M-22 cells) and express abundant levels of Sp1 (26). We found that cytoplasmic histone-associated internucleosomal DNA fragmenta-

FIG. 3. CAM stimulates apoptosis in vascular SMCs and induces FasL expression in an Sp1-dependent manner. A, CAM increases propidium iodide nuclear staining in WKY12-22 cells. The SMCs were exposed to CAM (1 μg/ml) for 24 h prior to fixation, incubation with propidium iodide, and confocal microscopy. Effect of CAM (1 μg/ml) on luciferase expression driven by FasLhsαLuc or a construct bearing a deletion of the Sp1 binding element (−271FasLhsαLuc, B), or FasLhsαLuc cotransfected with 5 μg of dominant-negative Sp1 (pEBGNLS-Sp1) or the backbone alone (pEBGNLS), C. 15 μg of FasL promoter-reporter construct was used throughout. Firefly luciferase activity was normalized to Renilla activity, and the results were plotted as -fold increase relative to FasLhsαLuc or −271FasLhsαLuc, respectively, in B, or the pEBGNLS or pEBGNLS-Sp1 groups in C. Error bars represent S.E. The data are representative of two independent determinations.

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Sp1 Phosphorylation Regulates FasL-dependent Apoptosis

Fig. 4. Phosphorylation of endogenous Sp1 following exposure to CAM. A, EMSA using [32P]FasL Oligo and nuclear extracts of WKY12-22 cells incubated with CAM (1 μg/ml). Where appropriate, the extracts were treated with CIP prior to EMSA. The amount of CIP (5 milliunit) used in this assay is based on CIP titration experiments that previously defined the concentration of CIP unable to dephosphorylate the 32P-labeled probe. Sp1 nucleoprotein complex intensity (with or without CAM exposure for CIP treatment of extracts) was semi-quantitated by densitometry. B, Western blot analysis using nuclear extracts of WKY12-22 cells exposed to CAM (1 μg/ml), with and without CIP treatment (5 units). Sp1-P indicates hyperphosphorylated Sp1. The Coomassie Blue-stained gel is shown. The data are representative of two independent determinations.

FaslhsLuc blocked CAM-inducible FasL transcription (Fig. 3C), whereas the empty expression vector had no effect (Fig. 3C). These data demonstrate that Sp1 is required for FasL promoter activation by extracellular stimuli.

EMSA using [32P]FasL oligonucleotide and nuclear extracts of cells exposed to CAM revealed that this agent did alter Sp1 occupancy of the promoter (Fig. 4A, lane 4 versus lane 2). Incubation of these extracts with CIP (22), which hydrolyzes 5'-phosphate groups, prior to EMSA decreased the intensity of both Sp1 binding complexes (Fig. 4A, lane 5 versus lane 3) but was mostly profound in extracts of cells exposed to CAM. Densitometric assessment of the intensities of these complexes (Fig. 4A, lower left and lower center) revealed that 12% of promoter-bound Sp1 is basally phosphorylated and that Sp1 phosphorylation increases to 31% upon exposure to CAM (Fig. 4A, lower right). This indirect determination of Sp1 phosphorylation was supported by Western immunoblot analysis with antibodies to Sp1. We observed the appearance of a hyperphosphorylated species following exposure to CAM (Fig. 4B). This effect was abolished by prior incubation of the extracts with CIP (Fig. 4B). These findings thus show that Sp1 is phosphorylated during CAM-inducible apoptosis. Sp1 phosphorylation regulates the
inducible expression of a number of other genes, including vascular permeability factor/vascular endothelial growth factor (31), \( \alpha_2 \)-integrin (35), and tissue factor (23).

CAM-inducible FasL Promoter Activity and Apoptosis Are PKC-\( \zeta \)-dependent Processes—A kinase found to mediate Sp1 phosphorylation is protein kinase-\( \zeta \) (PKC-\( \zeta \)) (31), a diacylglycerol- and \( Ca^{2+} \)-independent atypical member of the PKC family (36). PKC-\( \zeta \) is ubiquitously expressed and interacts directly with Sp1 (31). To investigate the role of PKC-\( \zeta \) in the regulation of FasL expression, we cotransfected WKY12-22 cells with an expression vector (CMV-FLAG-DN-PKC-\( \zeta \)) generating a kinase-inactive dominant-negative mutant of PKC-\( \zeta \) bearing a Lys\( ^{275} \)→Trp\( ^{275} \) substitution (37–40). The FasL promoter was activated by CAM in cells harboring the empty expression vector (Fig. 5A), whereas overexpression of mutant PKC-\( \zeta \) attenuated CAM-inducible FasL promoter-dependent reporter expression (Fig. 5A). Dominant-negative PKC-\( \zeta \) also blocked internucleosomal DNA fragmentation as a proportion of the total number of cells in the treatment population (Fig. 5B). Overexpression of dominant negative PKC-\( \zeta \) in cells not exposed to CAM did not significantly modulate the level of apoptosis compared with cells transfected with the backbone control (data not shown). This suggests that the capacity of dominant-negative PKC-\( \zeta \) to suppress apoptosis detectable in our system is conditional upon the cells being induced to undergo further cell death by exposure to apoptotic stimuli. This is likely a direct consequence of the low level of spontaneous phosphorylation of Sp1 (Fig. 4A) making attenuation by dominant-negative PKC-\( \zeta \) difficult to measure in a cotransfection setting. These findings, nonetheless, indicate that PKC-\( \zeta \) regulates inducible FasL transcription and apoptosis.

Fas receptor, unlike FasL (Fig. 1C), is expressed in both WKY12-22 and WKY3M-22 cells (Fig. 6A). Because CAM induces FasL expression (Figs. 3, B–D, and 5A), we hypothesized that the induction of apoptosis by this agent involves the secretion and autocrine/paracrine engagement of FasL with Fas at the cell surface. To address this possibility, prior to the addition of CAM, we incubated the cells with Fas\( ^{z} \)Fc chimera, in which the extracellular domain of Fas is fused to the Fc portion of human IgG. Fas\( ^{z} \)Fc blocked SMC apoptosis induced by CAM (Fig. 6B). In contrast, an identical amount of the Fc fragment alone had no effect (Fig. 6B). These findings thus demonstrate that autocrine/paracrine extracellular Fas/FasL engagement is involved in SMC apoptosis. Sp1 is phosphorylated and activates FasL in SMCs upon exposure to extracellular...
In this paper, we have defined a novel role for the ubiquitously expressed transcription factor Sp1 in apoptotic signal transduction. Subtypes of SMC expressing abundant levels of Sp1 produce FasL and undergo greater spontaneous apoptosis. EMSA and transient transfection analysis revealed that the FasL promoter is activated by Sp1 via a distinct element whose integrity is crucial for inducible expression. Inducible FasL transcription is inhibited by a mutant form of Sp1, which also blocks apoptosis. Inducible SMC apoptosis is preceded by Sp1 phosphorylation, increased FasL transcription, and the autocrine/paracrine engagement of FasL with Fas. Both inducible FasL transcription and apoptosis are blocked by dominant-negative protein kinase C-ζ. These data demonstrate that apoptotic signaling in SMCs involves Sp1 phosphorylation.

The present study is the first report of transcription factor phosphorylation as a prerequisite biochemical process in inducible apoptotic cell death. We used CAM as a model effector of cell death; however, given the general cellular expression of Sp1, our observations are unlikely to be confined to this agent alone nor are they likely to be cell type-specific. Okadaic acid, a selective inhibitor of serine-threonine phosphatases PP2A, stimulates apoptosis in a wide variety of cell types including murine fibroblasts (41), rat kidney epithelial cells (42) amongst others. Okadaic acid, like CAM, stimulates Sp1 phosphorylation and apoptosis in SMCs (data not shown). Tat, the transcriptional activator of human immunodeficiency virus type 1 (HIV-1), stimulates Sp1 phosphorylation (43), activates FasL expression (44), and can induce apoptosis (44, 45). Sp1 phosphorylation may be an important theme in apoptotic signaling. However, this process alone may not account for FasL transactivation because Sp1 physically interacts and functionally cooperates with a large number of other transcription factors. These include NF-κB p65/RelA (46, 47) and AP1 (48), which each induce the FasL promoter in other cell types (16, 19).

A common pathophysiological setting in which Sp1 phosphorylation may be relevant is atherosclerosis. Sudden death in patients with unstable angina and myocardial infarction is associated with atherosclerotic plaque rupture (49, 50). This could arise from SMC apoptosis, because SMCs are the only cells in the plaque capable of producing collagen fibers types I and III, which maintain tensile strength (8–10, 12, 51). Loss of SMCs as a consequence of apoptosis could weaken the cap and result in plaque rupture (12). Consistent with this, SMCs located in vulnerable regions of the plaque, such as the fibrous cap and shoulders (52, 53) undergo apoptosis (56, 54) and express Fas (5, 7). Indeed, SMCs isolated from atherosclerotic plaques undergo greater spontaneous apoptosis than cells derived from the normal artery wall (55). Moreover, SMC depletion in human aortic aneurysms is accompanied by biochemical and morphological changes consistent with SMC apoptosis (56). Sp1 phosphorylation may thus be an important biochemical mediator of cell death in a number of vascular disease settings.

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