Biomechanically Induced Gene iex-1 Inhibits Vascular Smooth Muscle Cell Proliferation and Neointima Formation


Abstract—Mechanotransduction plays a prominent role in vascular pathophysiology but is incompletely understood. In this study, we report the biomechanical induction of the immediate early response gene iex-1 in vascular smooth muscle cells (SMCs). Mechanical induction of iex-1 was confirmed by Northern (30-fold induction after 2 hours) and Western (6-fold induction after 24 hours) analyses. Expression of iex-1 was regulated by mechanical activation of nuclear factor (NF)-κB and abolished by overexpression of IκB in SMCs. The function of iex-1 in SMCs was explored by gene transfer using adenoviral vectors overexpressing iex-1. After 48 hours of 4% cyclic mechanical strain, adenoviral vectors overexpressing iex-1-infected cells had lower 3[H]-thymidine incorporation compared with AdGFP-infected controls (71.3±8.5% versus 180.2±19.4% in controls; P<0.001). Overexpression of iex-1 suppressed mitogenesis induced by platelet-derived growth factor (208.1±108.3% versus 290.0±120.5% in controls; P<0.05). This was accompanied by reduced degradation of p27kip1, inhibition of Rb hyperphosphorylation, and reduced cell cycle progression. To investigate functional effects of iex-1 in vivo, we performed carotid artery mechanical injury and endothelial denudation in low-density lipoprotein receptor−deficient mice followed by intraluminal injection of adenoviral vectors (3×10⁹ pfu in 50 μL) for overexpression of iex-1 or gfp (control). Vascular overexpression of iex-1 reduced neointima formation 2 weeks after injury (intima/media ratio, 0.23±0.04 versus 0.5±0.24 in controls; P<0.05). Our findings demonstrate that biomechanical strain induces iex-1 with subsequent antiproliferative effects in SMCs and that selective gene transfer of iex-1 inhibits the local vascular response after injury. These findings suggest that the induction of iex-1 represents a novel negative biomechanical feedback mechanism limiting the vascular response to injury. (Circ Res. 2003;93:1210-1217.)

Key Words: atherosclerosis | gene expression | growth factors | smooth muscle cells

Mechanotransduction plays a central role in the highly coordinated cellular response cascades of the vasculature. Transduction of biomechanical signals leads to activation of cellular signaling mechanisms, such as an increase in intracellular calcium, activation of stress-response protein kinases, and activation of transcription factor nuclear factor (NF)-κB. These signaling mechanisms ultimately lead to adaptive, and sometimes maladaptive, changes in cells and tissues. In vascular smooth muscle cells (SMCs), a well-documented response to biomechanical stimulation is cell proliferation. The mitogenic activation of SMCs participates in the local vascular reaction to hypertension as well as late lumen loss and restenosis after vascular interventions. Mechanically induced SMC proliferation is, at least in part, mediated by secretion of proteins with proliferative effects, such as platelet-derived growth factor (PDGF) and fibroblast growth factor-2 in the vascular wall after injury. Biomechanical signals induce a highly restricted transcriptional response in vascular SMCs that includes genes that can modify vascular structure.

iex-1 is a NF-κB response gene that regulates cellular growth and differentiation of neoplastic and nonneoplastic cells. This gene inhibits growth and can also promote apoptosis in some circumstances. The promoter region of iex-1 contains functional binding sites for p53 and NF-κB complexes. In cardiomyocytes, iex-1 is regulated by NF-κB and exhibits antihypertrophic effects. In isolated human monocytes, iex-1 is highly expressed but undergoes rapid suppression during cellular differentiation. In addition, biomechanical stimulation increases the expression of iex-1 in monocytes. It has been demonstrated that IEX-1 interacts with extracellular signal kinases (ERK1/2), inhibits Akt activation, and attenuates NF-κB activation; however, the precise function of IEX-1 is incompletely understood.
In this study, we report the robust induction of the early response gene *iex-1* by biomechanical stimuli under the transcriptional control of NF-κB activation. Adenoviral gene transfer of *iex-1* mediates distinct antiproliferative effects in SMCs in vitro. Furthermore, *iex-1* is overexpressed in athrosclerosis and vascular gene transfer of *iex-1* inhibits neointima formation after pressure-induced carotid artery injury in vivo. Therefore, the biomechanical induction *iex-1* represents a novel negative feedback mechanism in response to vascular injury.

Materials and Methods

Cell Culture and Strain Experiments

Human aortic SMCs were isolated from surgical specimens and cultured with previously described methods. Cells were plated in 10% FCS in DMEM on fibronectin-coated silicone membranes that allow controlled biaxial strain (1% to 9%, 1 Hz). After 24 hours, cells were washed with PBS and serum-starved for 48 hours in DMEM containing ITS supplement (Sigma) before experiments were initiated. In addition, cells were stimulated with PDGF-BB (4 ng/mL), tumor necrosis factor-α (TNF-α, 10 ng/mL), interleukin-1β (IL-1β, 10 ng/mL), angiotensin II (10 nmol/L), or phorbol 12-myristate 13-acetate (PMA, 100 ng/mL). For experiments using adenoviral gene transfer of *IEX-1* or green fluorescent protein (GFP) (controls), cells were infected with adenoviruses at a multiplicity of infection of 200 (200 infectious particles per cell for the infection of >99% of the cells). After 6 hours, cells were washed in PBS and incubated for another 36 hours in DMEM with ITS. Infection efficiency was confirmed by GFP fluorescence.

Northern Analysis

For the detection of mRNA transcripts of *IEX-1* by Northern analysis, a cDNA probe was synthesized using the following oligonucleotides: 5′-TAACCCACTCCACCCATGA-3′ and 5′-GTTCAAAGCTCGAGGA-3′. Total RNA was isolated, and identical amounts of RNA were loaded on a 1% agarose gel containing formaldehyde. After transfer to membranes, the membranes were incubated with radiolabeled probes.

Western Analysis

Protein expression was analyzed by Western blotting using a polyclonal rabbit anti-*IEX-1* antibody. Additional immunoblotting was performed using specific antibodies against ERK1/2 (Santa Cruz Biotechnology), phospho-p44/p42 mitogen-activated protein kinase (ERK1/2; Cell Signaling Technology), p27Kip1, and Rb (BD Biosciences). After incubation with a horseradish peroxidase–conjugated secondary antibody, bands were visualized by chemiluminescence (Perkin Elmer).

Electrophoretic Mobility Shift Assay

Nuclear extracts were prepared as described before. NF-κB–specific oligonucleotides (Promega) were end-labeled using T4 polynucleotide kinase and [γ-32P]ATP (NEN). Nuclear extracts were incubated for 10 minutes in binding buffer followed by 20 minutes of incubation with labeled oligonucleotide. Samples were separated on a 4% native polyacrylamide gel. For competition studies, 50× excess unlabeled oligonucleotide was used, and for the supershift assay, the nuclear extracts were incubated with 2 μg of anti-p50 or anti-p65 antibody (Santa Cruz).

Recombinant Adenoviral Gene Vectors

Recombinant construction of adenoviral vectors was performed as described before. The recombinant IxkB adenovirus (AdIxkB) expressing the porcine 1xβ-gene was a gift from Dr Josef Amrath (Beth Israel Deaconess Medical Center, Boston, Mass.).

Methyl-[H]-Thymidine Incorporation

Cellular proliferation was detected by incorporation of methyl-[H]-thymidine during de novo DNA synthesis. Five hours before termination of the experiments, methyl-[H]-thymidine (6.7 μCi/mmol) was added to the culture medium (final concentration, 2 μCi/mL). Cells were washed with ice-cold PBS (pH 7.4), followed by incubation in 10% trichloracetate at 4°C. Precipitates were solubilized in 0.2 N NaOH at 37°C and analyzed in a liquid scintillation chamber for radioactive incorporation. Cellular proliferation was expressed as relative amounts of radioactive incorporation (counts per minute) as percentage of baseline incorporation.

Flow Cytometry

For cell cycle analysis, cells were infected with adenoviral vectors for overexpression of *IEX-1* and GFP (AdIEX-1) or GFP alone (AdGFP). After 48 hours, cells were washed with PBS, harvested, and fixed in 80% ethanol in PBS at −20°C. Afterward, cells were centrifuged and resuspended in 1 mL of PBS. RNAse H (Sigma) was added for digestion of RNA (30 minutes). Cellular DNA content was detected by propidium iodide (100 μg/mL; Sigma) and analyzed using a Cytomics FC 500 flow cytometer (Beckman Coulter). GFP-positive cells were gated, and the percentage of cells in a specific cell cycle phase (G0/G1, S, and G2/M) were determined based on peak signal intensity according to the internal protocol of the flow cytometer (Beckham Coulter).

Expression of IEX-1 in Atherosclerotic Lesions of Apolipoprotein E−/− Mice

Apolipoprotein E–deficient (ApoE−/−) mice were fed a high-cholesterol diet (1.25% cholesterol, Purina) for 10 weeks (n=6). ApoE−/− mice under low-cholesterol diet (n=4) served as controls. After euthanasia, the carotid arteries were removed, fixed in 4% paraformaldehyde, and embedded in paraffin for additional histological analysis.

Human Carotid Atheroma Specimens

Samples of human carotid atheromas were obtained from patients undergoing surgical endarterectomy. The tissue was frozen in liquid nitrogen immediately after removal and stored at −80°C. Serial cryostat sections (5 μm) were cut, air dried onto microslide slides, and fixed in acetone (−20°C, 5 minutes) before subsequent histological analysis.

Vascular Gene Transfer and Carotid Injury Model in LDL-Receptor−/− Mice

LDL receptor−/− mice (mean age, 18 weeks; Jackson Laboratories, Bar Harbor, Maine) were fed a high-cholesterol diet (1.25% cholesterol, Purina) for 10 weeks before vascular injury of the right carotid artery was performed. The animals were anesthetized (75 mg/kg ketamine and 7 mg/kg xylazine in 0.9% saline solution IP), followed by surgical exposure of the right carotid artery. An incision was made, followed by insertion of a 30-gauge blunted needle that was fixed to the external carotid artery. The isolated, saline-filled common carotid segment was dilated with 1.5 atm of pressure for 90 seconds using an angioplasty inflation device (Advanced Cardiovascular Systems/Guidant). Endothelial denudation was performed by air drying for 10 minutes (20 mL/min). Next, the artery was exposed to adenoviral vectors (3×1010 pfu in 50 μL of 10 mmol/L Tris, pH 8.0, with 2 mmol/L MgCl2 and 4% sucrose) for 30 minutes. Afterward, the needle was removed, and the external artery was ligated proximal to the insertion site. The air exit hole in the common carotid artery was closed, and the incision was closed with 4-0 silk sutures. Vascular gene transfer was confirmed by overexpression of GFP in the vessel wall on selected animals 48 hours after surgery. The animals were euthanized 14 days after vascular injury. For comparison of injured versus noninjured vessels, both carotid arteries were removed for histological analysis. All animals received bromodeoxyuridine (50 mg/kg IP) 18 hours and 1 hour before
Biomechanical Strain Induces the Expression of iex-1 in SMCs

Cultured SMCs were exposed to different amplitudes of mechanical strain for the analysis of iex-1 expression. Expression of iex-1 in SMCs showed strong amplitude dependency, with maximal induction at 4% cellular strain (30-fold induction after 2 hours; \( P<0.0001 \)) (Figure 1A). The expression of iex-1 was induced 30 minutes after the onset of 4% mechanical strain (1 Hz) and reached a maximum after 2 hours (Figure 1B). The time-dependent induction of iex-1 expression was confirmed on the protein level, reaching a maximum after 12 hours of mechanical strain (6-fold induction after 24 hours; \( P<0.001 \)) (Figure 1C). These data identify iex-1 as a mechanically inducible gene in SMCs.

Because the cellular response cascade to mechanical strain includes the expression and secretion of vascular growth factors and cytokines, we investigated the regulation of iex-1 by neurohumoral factors involved in vascular proatherogenic mechanisms. Baseline expression of iex-1 in serum-starved SMCs increased after incubation with DMEM containing 10% FCS. Stimulation with IL-1β, PDGF, and PMA strongly induced iex-1 expression after 2 hours (Figure 1D). In contrast, stimulation with TNF-α and angiotensin II induced only a marginal increase in iex-1 expression after 24 hours. The growth factor PDGF time-dependently induced the expression of iex-1 in SMCs, with maximal induction after 6 hours (32-fold induction after 6 hours; \( P<0.0001 \)) (Figure 1E). Therefore, in addition to biomechanical strain, iex-1 expression is regulated by vascular neurohumoral factors, including PDGF and the proinflammatory cytokine IL-1β.

Identification of iex-1 as an NF-κB–Regulated Gene in SMCs

The transcription factor NF-κB has been implicated in the coordinated response cascades after vascular injury and inflammation. We investigated the role of NF-κB in the transcriptional regulation of iex-1 expression in SMCs. Mechanical strain of 4% resulted in early and sustained activation of NF-κB in SMCs (Figure 2A). Supershift analysis revealed the involvement of p50 and p65 in strain-induced activation of NF-κB (Figure 2B). To investigate whether the strain-induced activation of NF-κB is a necessary event for the induction of iex-1 expression, we overexpressed IκB, the cytoplasmic binding partner and negative regulator of NF-κB activation, in SMCs. Cell overexpressing GFP served as controls. Although no differences in iex-1 expression were detectable at baseline, the mechanical induction of iex-1 was abolished in cells overexpressing IκB. These findings identify activation of NF-κB as a necessary event in the biomechanical induction of iex-1 in SMCs.
Adenoviral Gene Transfer of iex-1 Inhibits the Proliferation of SMCs

To investigate functional effects of iex-1 in vitro, SMCs were infected with adenoviral constructs for selective gene transfer of iex-1 with gfp (AdIEX-1) or gfp alone (AdGFP, controls). Adenoviral gene transfer resulted in a robust overexpression of iex-1 protein in SMCs (Figure 3A). Stable infection of >99% of the cells identified by positive GFP fluorescence was seen after 48 hours, without morphological differences of cells infected with AdIEX-1 compared with AdGFP (Figure 3B).

Mechanical strain induces a well-characterized proliferative response in cultured SMCs. Therefore, we analyzed functional effects of IEX-1 on the proliferation of SMCs exposed to mechanical strain. At baseline, adenoviral gene transfer of iex-1 did not affect \( [H]\)-thymidine incorporation compared with AdGFP-infected controls. No significant changes in cellular proliferation were detectable after 24 hours of 4% cyclic mechanical strain in AdGFP-infected or AdIEX-1-infected cells. However, 48 hours of 4% strain induced cellular proliferation, as detected by an increased \( [H]\)-thymidine incorporation in noninfected and AdGFP-infected cells (AdGFP-infected cells, 180.2 \pm 19.4% of baseline; \( P < 0.001 \)). Adenoviral overexpression of iex-1 abolished the strain-induced increase in \( [H]\)-thymidine incorporation after 48 hours compared with AdGFP-infected SMCs (71.3 \pm 8.5% in AdIEX-1–infected cells versus 180.2 \pm 19.4% in AdGFP-infected cells; \( P < 0.0001 \)) (Figure 3C).

Next, we explored the role of iex-1 in strain-independent mechanisms of cellular proliferation that were induced by incubation of SMCs with the vascular growth factor PDGF (4 ng/mL for 24 hours). At baseline, no significant differences in \( [H]\)-thymidine incorporation were observed between cell overexpressing iex-1 and controls (AdIEX-1–infected cells, 95.9 \pm 36.2% of nonstimulated AdGFP-infected cells). Stimulation with PDGF for 24 hours induced cellular proliferation in AdGFP-infected SMCs, detected by increased \( [H]\)-thymidine incorporation (290.0 \pm 120.5% compared with nonstimulated cells; \( P < 0.0001 \)). Gene transfer of iex-1 reduced PDGF-induced cellular proliferation compared with AdGFP-infected controls (208.1 \pm 108.3% in AdIEX-1–infected cells versus 290.0 \pm 120.5% in AdGFP-infected cells; \( P < 0.05 \)) (Figure 3D). Altogether, these data demonstrate antiproliferative effects of iex-1 in SMCs that inhibit cellular proliferation both in response to mechanical strain and the mitogenic growth factor PDGF.
Inhibition of Cell Cycle Progression by IEX-1

To investigate the underlying mechanisms for antiproliferative effects of iex-1 in SMCs, we assessed PDGF-induced mechanisms of cellular proliferation in SMCs. Cells were infected with AdIEX-1 or AdGFP and serum-starved for 72 hours, followed by stimulation with PDGF for 24 hours. At baseline, no differences in cellular levels of the Cdk inhibitor p27kip1 and hyperphosphorylation of Rb, two central regulators of mitotic events in SMCs, were detectable. Stimulation of SMCs with PDGF for 24 hours induced degradation of p27kip1 in AdGFP-infected controls, which was inhibited by overexpression of iex-1. Moreover, PDGF-induced hyperphosphorylation of Rb was detectable in AdGFP-infected cells, whereas cells infected with AdIEX-1 showed a significant reduction in hyperphosphorylated Rb (Figure 4A). Recently, regulation of ERK1/2 by IEX-1 has been reported in a human megakaryoblastic cell line (UT7). Therefore, we assessed levels of total ERK in SMCs after adenoviral gene transfer of iex-1. However, no differences in cellular levels of total ERK1/2 at baseline or after stimulation with PDGF were detectable in SMCs infected with AdIEX-1 or AdGFP. Moreover, the PDGF-induced phosphorylation of ERK1/2 was not altered in cells overexpressing iex-1 compared with AdGFP-infected cells (Figure 4B).

Next, we investigated stages of cell cycle progression in SMCs infected with AdIEX-1 or AdGFP after stimulation with PDGF. At baseline, no differences in the percentage of cells in G0/G1 arrest, S, or G2/M phase were detectable (G0/G1, 84.3±1.8% versus 86.4±1.3%; S, 5.9±1.0 versus 4.9±0.6; G2/M, 8.9±1.0 versus 7.8±0.7 in AdIEX-1–infected cells, respectively; P=NS). As expected, stimulation with PDGF for 24 hours induced cell cycle progression in AdGFP-infected SMCs (G0/G1, 77.0±2.3%; S, 7.0±1.8%; G2/M, 14.9±0.3; P<0.01 versus nonstimulated controls). In contrast, overexpression of iex-1 blocked cell cycle progression and DNA replication (G0/G1, 84.6±1.3%; S, 5.9±0.9%; G2, 9.0±0.9%; P<0.001 versus AdGFP-infected cells) (Figures 4C and 4D). These findings indicate an inhibition of cell cycle progression by overexpression of iex-1 in SMCs, which is consistent with the inhibition of [3H]thymidine incorporation after adenoviral gene transfer of iex-1.

Enhanced Expression of iex-1 in Atherosclerosis

ApopE–/– mice on a high-cholesterol diet for 10 weeks exhibited enhanced expression of iex-1 with distinct localization in the media of carotid arteries and weak immunohistochemical staining of the endothelium. Atherosclerotic plaques of animals on high-cholesterol diet stained weakly positive for iex-1. The surrounding adventitial tissue showed minimal immunoreactivity (Figure 5A). In addition, immunohistochemical detection of IEX-1 in human carotid atheroma specimens revealed strong immunoreactivity in the shoulder region of the atheroma and the vascular wall (Figure 5B). Immunohistochemical detection of NF-κB in atherosclerotic vessels did not reveal a clear colocalization of IEX-1 with

Figure 4. Overexpression of iex-1 inhibits cell cycle progression in SMCs. A, Overexpression of iex-1 inhibited the PDGF–induced degradation of p27kip1 compared with GFP-infected cells. In addition, PDGF–induced hyperphosphorylation of Rb was significantly reduced in cells infected with AdIEX-1 but not affected by overexpression of gfp. B, Overexpression of iex-1 or gfp did not change levels of total ERK1/2 at baseline or after stimulation with PDGF. PDGF–induced phosphorylation of ERK1/2 showed no significant difference in AdIEX-1–infected compared with AdGFP–infected cells. C, Using flow cytometry for cell cycle analysis, no differences between AdGFP–infected and AdIEX–infected cells were detected in quiescent SMCs. D, Stimulation with PDGF (4 ng/mL) resulted in cell cycle entry and progression in AdGFP–infected SMCs that was not detectable in AdIEX–infected cells. After stimulation with PDGF, a significantly higher number of AdIEX–infected cells remained in G0/G1 arrest compared with controls (P<0.001 vs AdGFP–infected cells). In addition, overexpression of iex-1 reduced the number of cells in G2/M phase after stimulation with PDGF compared with AdGFP–infected controls (P<0.001 vs AdGFP–infected controls). Mean data from 3 independent experiments.
activated NF-κB, indicating that other mechanisms are likely to be involved in the regulation of iex-1 expression in vivo (data not shown). IEX-1 colocalizes with CD68 in the shoulder region of the plaque in human atheroma sections, consistent with studies demonstrating expression of iex-1 in human macrophages.14 In addition, aortic SMCs show expression of IEX-1 in human atherosclerosis. Therefore, increased expression of iex-1 in atherosclerotic vessels of ApoE−/− mice and humans subjects implicates an involvement of iex-1 in the response mechanisms of vascular injury.

**Overexpression of iex-1 Inhibits Neointima Formation After Vascular Injury In Vivo**

We next investigated the functional effects of adenoviral-mediated gene transfer of iex-1 in a mouse model of carotid artery injury in vivo. Vascular injury was performed in LDL-receptor−/− mice on high-cholesterol (1.25%) diet for 10 weeks through air-pressure inflation and endothelial denudation of the right carotid artery. This procedure results in a well-characterized vascular response, with typical platelet adhesion, monocyte infiltration subsequently leading to neointima formation.18 We extended this procedure by intraluminal incubation with adenoviral vectors for 30 minutes for selective gene transfer of iex-1 (n=16) and gfp (n=14, controls).

In control animals infected with AdGFP, carotid artery injury induced robust neointima formation that resulted in an increased neointima/media ratio 2 weeks after the procedure (Figure 6A). Weak immunoreactivity for IEX-1 was detectable in the intimal hyperplasia of carotid arteries of AdGFP-infected animals. In contrast, the injury-induced neointima formation was inhibited by overexpression of iex-1 in the vascular wall (intima/media ratio, 0.23±0.04 in AdIEX-1–infected animals versus 0.5±0.24 in AdGFP-infected controls; P<0.05 [n=6 per group]) (Figure 6B). No differences in cellular content or monocyte infiltration were detectable between the two groups. These findings demonstrate the negative regulation of vascular response mechanisms after pressure-induced injury of the carotid artery through inhibition of neointima formation by adenoviral overexpression of iex-1.

**Discussion**

Mechanotransduction plays a pivotal role in cellular growth and differentiation and in remodeling tissues.1 In the vasculature, it has been identified as an essential factor maintaining vessel wall structure and function.5 However, mechanotransduction also participates in proatherogenic mechanisms in hypertension and after vascular injury.4,5,19

In this study, the early response gene iex-1 was identified as a biomechanically controlled and cytokine-inducible gene in human vascular SMCs. Mechanical induction of iex-1 expression requires activation of the transcription factor NF-κB. Local overexpression of iex-1 was found in atherosclerotic vessels and human atheroma specimens with distinct localization in the vascular media and the shoulder region of the plaque. In various experimental models, we identified antiproliferative effects of iex-1 in vitro and showed inhibitory effects of iex-1 on neointima formation after pressure-induced vascular injury through adenoviral gene transfer of this gene in vivo. These findings suggest that iex-1 participates in a negative feedback mechanism that may limit the local vascular response to injury.

iex-1 was described as a gene of unknown function expressed by stimulated fibroblasts20 and epithelial cells.21,22

**Figure 6.** Overexpression of iex-1 inhibits neointima proliferation in vivo. Vascular injury of the carotid artery was induced by pressure inflation followed by endothelial denudation. The denuded vessels were infected with 3×10^9 pfu of adenoviral constructs in 50 μL of buffer for selective overexpression of iex-1 or gfp (controls). A, Pressure-induced vascular injury results in luminal neointima formation in vessels infected with AdGFP that is accompanied by progressive vessel enlargement. Adenoviral gene transfer of iex-1 strongly inhibits neointima formation after vascular injury in vivo (Verhoeff van Giesson, ×20). B, Neointimal area was quantified on serial sections of the remodeling carotid arteries and expressed relative to media area of the vessels. Overexpression of iex-1 significantly inhibits neointima formation after vascular injury in vivo (neointima to media ratio, 0.25±0.1 vs 0.51±0.24; *P<0.05 vs AdGFP-infected animals [n=6 per group]).
Several transcription factors, such as p53, NF-κB, and SP1, control the transcriptional regulation of iew-1 gene expression. Recently, several studies have characterized functional effects of iew-1, describing enhanced activation of ERK, inhibition of Akt activation, and attenuation of NF-κB activation. However, its regulation and function seem to depend on distinct cofactors and cell type. iew-1 is highly expressed in several cancer cells, such as metastatic melanoma cells and breast cancer cells, and has been implicated in cell cycle regulation and cellular growth. In these in vitro models, selective overexpression of iew-1 inhibits cellular growth and mediates proapoptotic effects.

Activation of the transcription factor NF-κB is a central event in the vascular response cascades after local injury and inflammation and in atherosclerosis. NF-κB activation by biomechanical transduction processes has been described in cardiomyocytes as well as in vascular SMCs. Furthermore, NF-κB can be activated by several proinflammatory cytokines in SMCs. The present study identifies iew-1 as an NF-κB-controlled gene in SMCs in vitro, which is consistent with previous reports on the transcriptional control of iew-1 in cardiomyocytes. Functional effects of NF-κB activation involve the local expression of proinflammatory cytokines, SM accumulation, and oxidative stress. The NF-κB dependent induction of iew-1, therefore, may represent a negative feedback mechanism in the vascular response to injury.

Our previous experiments suggest that biomechanical strain induces a highly restricted transcriptional response. The present study describes the inhibition of SMC proliferation after adenoviral gene transfer of the strain-induced gene iew-1 and its relevance for processes of cellular growth and differentiation. The antiproliferative effects of iew-1 seem to be independent of the specific mitogenetic stimulus, because overexpression of iew-1 inhibits DNA de novo synthesis, both in response to mechanical strain and PDGF. Both stimuli induce cellular proliferation by different intracellular signal cascades. Thus, the nonselective inhibition of cellular proliferation implicates an arrest or stabilization of cell cycle progression by iew-1, consistent with participation of this gene in cell cycle regulation. This hypothesis is supported by our finding that adenoviral overexpression of iew-1 results in an inhibition of cell cycle progression in vascular SMCs. Furthermore, overexpression of iew-1 inhibits growth factor-induced degradation of the Cdk inhibitor p27kip1 and hyperphosphorylation of Rb. Intriguingly, both molecules have recently been involved in stretch-induced activation of SMC proliferation.

Our in vivo experiments demonstrate an enhanced expression of iew-1 in atherosclerotic vessels of ApoE−/− mice on high-cholesterol diet and in human atheroma specimens. Even though our in vitro experiments reveal a central role of transcription factor NF-κB in the regulation of iew-1 expression, immunohistochemical colocalization studies did not reveal a clear relationship between NF-κB and iew-1 staining, so that other mechanisms likely also regulate iew-1 expression. iew-1 is expressed in SMCs of the vascular media detected by SMC actin and exhibits distinct colocalization with the macrophage marker CD68 in the shoulder region of the plaque. Therefore, we conclude that expression of iew-1 is regulated in atherosclerosis and might participate in vascular response mechanisms. This is additionally supported by the functional effects of iew-1 on neointima formation after vascular injury. The inhibition of neointima formation by overexpression of iew-1 underlines the role of iew-1 as a negative regulator of proliferative mechanisms after vascular injury. Overexpression of this biomechanically induced gene might, therefore, sustain an important endogenous mechanism that maintains vessel wall morphology and function after localized injury of the vessel wall.

Presently, the molecular mechanisms by which iew-1 regulates cellular growth are incompletely understood. Analysis of the iew-1 protein sequence has not yet been revealing, and the sequence of iew-1 suggests that it is a highly disordered protein that may function through binding to other proteins (authors’ unpublished observations, 2003). Furthermore, molecular studies will be required to elucidate proteins that directly associate with iew-1.

A limitation of the present study is the unknown function of iew-1 in atherosclerosis. In addition, additional analysis of the sequential expression of iew-1 after vascular injury could provide more insights into the functional role of iew-1 in the vascular response cascades. Many genes active in developing blood vessels are reinduced in vascular lesions; therefore, it will be interesting to investigate iew-1 expression in developing tissues. Additional studies should also clarify the specific function of iew-1 in cell cycle regulation and investigate its specific molecular mechanisms. Finally, sequential analysis of iew-1 expression in SMC proliferation and its relation to the expression of specific SMC differentiation markers regulated by paracrine factors would help to define what type of SMCs express iew-1 and may provide more insight into the functional role of iew-1.

In conclusion, our findings show the regulation of the early response gene iew-1 by mechanical strain and growth factors in human vascular SMCs. An important function of this gene may be its antiproliferative effects in SMCs in response to several stimuli. However, it is possible that the induction of iew-1 in the presence of mechanical strain and serum-derived factors represents a mechanically induced phenotypic conversion of cultured SMCs toward a more physiological phenotype. This is consistent with basal expression of IEX-1 detected by immunohistochemistry in native, noninjured vessels and the early but not persistent expression of iew-1 after vascular injury, as demonstrated in the present study. We hypothesize that the induction of iew-1 represents a negative feedback mechanism in vascular SMCs that limits the local vascular response in atherosclerosis and after vascular injury.

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