Small Mechanical Strains Selectively Suppress Matrix Metalloproteinase-1 Expression by Human Vascular Smooth Muscle Cells

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Mechanical forces and biochemical stimuli may interact to regulate cellular responses. In this study, we tested the hypothesis that very small mechanical strains interact with growth factors in the regulation of matrix metalloproteinase (MMP)-1. Human vascular smooth muscle cells (VSMCs) were cultured on a precoated silicone membrane in a device that imposes a highly uniform biaxial strain. VSMCs cultured on fibronectin were treated with cyclic 1-Hz strains of 0, 1, or 4%, and MMPs were assayed by Western analysis or gelatin zymography. Small strains did not induce MMP-1 in VSMCs, but strain was a potent inhibitor of platelet-derived growth factor (PDGF)- or tumor necrosis factor-α-induced synthesis of MMP-1. In contrast, MMP-2 and TIMP-2 levels were not changed by PDGF and/or mechanical strain. VSMCs strained on the 120-kDa chymotryptic fragment of fibronectin or RGD peptides suppressed PDGF-induced expression of MMP-1, indicating that this effect is not mediated by the heparin-binding domain or connecting segment-1 of fibronectin. Northern analysis of *ets-1*, a transcriptional activator of MMP-1 expression, showed that strain down-regulated *ets-1* expression, whereas *c-fos* expression was augmented. Thus, small deformations can selectively suppress MMP-1 synthesis by VSMCs, demonstrating the exquisite sensitivity of the cell to mechanical stimuli.

In the vessel wall as well as in other tissues, adaptation occurs through constant cellular migration, proliferation, and death and extracellular matrix synthesis and degradation. In stable normal tissues, the rates of these processes are so slow that they may appear almost dormant, whereas in injured or repairing tissues, the processes may all be greatly accelerated. In any wound repair, it is critical that the repaired tissue be sufficiently strong to withstand mechanical forces the tissue may experience. However, while the importance of biochemical mediators such as cytokines and growth factors in tissue repair and homeostasis is clear (1–3), how mechanical forces may interact with growth by mechanical strain (4–6). Wilson et al. (7, 8) have demonstrated that mechanical strain stimulates a mitogenic response in rat VSMCs through induction of platelet-derived growth factor (PDGF), and this induction is regulated by specific extracellular matrix interactions. Thus, VSMCs may sense mechanical stimuli (mechano-transduction) and change arterial structure.

Several studies have shown that mechanical forces exert important regulatory effects on vascular smooth muscle cells (VSMCs), including increased collagen synthesis and cell growth by mechanical strain (4–6). Wilson et al. (7, 8) have demonstrated that mechanical strain stimulates a mitogenic response in rat VSMCs through induction of platelet-derived growth factor (PDGF), and this induction is regulated by specific extracellular matrix interactions. Thus, VSMCs may sense mechanical stimuli (mechanotransduction) and change arterial structure.

One way VSMCs can change arterial structure is through the matrix metalloproteinases (MMPs). The MMPs are members of a family of enzymes that digest specific components of the extracellular matrix and may play a critical role in tissue repair and remodeling. The enzymatic activity of MMPs is regulated at several levels, including transcription; for example, cytokines such as IL-1, TNF-α, and PDGF induce secretion of MMP proenzymes (9, 10). These latent proenzymes can then be activated in the extracellular space (11, 12). Finally, the active MMPs may be inhibited by TIMPs (tissue-type inhibitors of matrix metalloproteinase), specific endogenous inhibitors of the MMPs. Several MMP promoters contain the 12-O-tetradecanoylphorbol-13-acetate response element, which binds AP-1, transcription factor dimeric combinations of c-Fos and c-Jun, as well as polyoma enhancer activator sites (PEA3), which bind the Ets family of transcription factors (13, 14). Several recent lines of evidence suggest that mechanotransduction through the extracellular matrix may regulate secretion of MMPs. First, James et al. (15) reported that direct mechanical wounding of a cellular monolayer of VSMCs induces MMP expression. Second, perturbing the cytoskeleton of rabbit synovial fibroblasts induces MMP expression (16). Third, Huhtala et al. (17) have demonstrated that domains of fibronectin (FN) may interact with different integrin subunits to either induce or suppress collagenase (MMP-1) expression in rabbit synovial fibroblast cells. Finally, we and others (18, 19) have observed that, in vivo, MMP-1 is overexpressed at sites of mechanical overload in the diseased artery.

Using a mechanical deformation device that applies a highly uniform biaxial strain field over the culture substrate, we explored the hypothesis that mechanical deformations regulate MMP-1 secretion by VSMCs. Surprisingly, we found that very small strains do not induce MMP-1, but abolish induction of MMP-1 by PDGF or TNF-α, demonstrating that mechanical stimuli may potentially interact with biochemical signals in regulating extracellular matrix metabolism.

The abbreviations used are: VSMCs, vascular smooth muscle cells; PDGF, platelet-derived growth factor; MMP, matrix metalloproteinase; IL-1, interleukin-1; TNF-α, tumor necrosis factor-α; FN, fibronectin; MEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum.

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membrane was incubated with 1:2000 diluted rabbit anti-human collagen type I (IRMA, New England Nuclear, Boston, MA). After 1 h, the membrane was rinsed three times with PBS and incubated with 50% nonfat dried milk in PBS for 1 h. The membrane was washed with PBS and incubated overnight at 4°C in antisense and control 32P-labeled oligonucleotides. The primer set for the synthesis of c-fos contained 5′-CTA-GGG-GTC-ATC-CTC-CCG-3′ and 5′-TAC-GGC-GTT-GGC-CTC-CTC-3′, yielding a 431-base pair cDNA. The probes were radiolabeled by the random priming method with [α-32P]dCTP and the Klenow fragment of DNA polymerase (Promega, Madison, WI). For Northern blotting, 15 μg of RNA was loaded on a 1.0% formaldehyde gel (2.0%) and transferred to a nylon membrane (ARAMCO Life Science). The gel was run at 200 V for 2 h. The membrane was baked at 70°C for 2 h and then hybridized with a 32P-labeled cDNA probe. The probe was hybridized with a peroxidase-labeled antibody (DakoCytomation, Carpinteria, CA). The membrane was then washed for 30 min at 65°C and incubated for 1 h with 1:4000 diluted rabbit anti-human TIMP-1 polyclonal antibody and 1:1000 diluted rabbit anti-human TIMP-2 polyclonal antibody (gift of Dr. R. A. Hynes, Harvard Medical School, Boston, MA). For the detection of TIMP-1 and TIMP-2, the membranes were blocked with 5% nonfat dried milk in TBS/Tween 20 for 1 h. The membrane was washed with TBS washing buffer (20 mM Tris base [pH 7.6], 137 mM NaCl, and 0.1% Tween 20) for 2 h. For the detection of collagenase 1 (MMP-1), the membrane was incubated with 1:2000 diluted rabbit anti-human collag- enase polyclonal antibody (generous gift of Merck Research Laboratories) for 1 h at 37°C and washed with TBS washing buffer for 30 min. The secondary antibody, goat anti-rabbit IgG coupled to peroxidase, was diluted 1:4000 and incubated with the membrane for 30 min. After washing with TBS washing buffer for 30 min, the membrane was developed by the enhanced chemiluminescence method (ECL, Amer- sham Life Science, Inc.). The gelatinolytic activity was then visualized by ethidium bromide staining. The membrane was incubated with 1:1000 diluted rabbit anti-human TIMP-1 polyclonal antibody and 1:1000 diluted rabbit anti-human TIMP-2 polyclonal antibody (gift of Dr. R. A. Hynes, Harvard Medical School) for 1 h at 37°C and washed with TBS washing buffer for 30 min. The secondary antibody, goat anti-rabbit IgG coupled to peroxidase, was diluted 1:4000 and incubated with the membrane for 30 min. After washing with TBS washing buffer for 30 min, the membrane was developed by the enhanced chemiluminescence method (ECL, Amer- sham Life Science, Inc.). The gelatinolytic activity was then visualized by ethidium bromide staining. The membrane was incubated with 1:1000 diluted rabbit anti-human TIMP-1 polyclonal antibody and 1:1000 diluted rabbit anti-human TIMP-2 polyclonal antibody (gift of Dr. R. A. Hynes, Harvard Medical School) for 1 h at 37°C and washed with TBS washing buffer for 30 min. The secondary antibody, goat anti-rabbit IgG coupled to peroxidase, was diluted 1:4000 and incubated with the membrane for 30 min. After washing with TBS washing buffer for 30 min, the membrane was developed by the enhanced chemiluminescence method (ECL, Amer- sham Life Science, Inc.). The gelatinolytic activity was then visualized by ethidium bromide staining. The membrane was incubated with 1:1000 diluted rabbit anti-human TIMP-1 polyclonal antibody and 1:1000 diluted rabbit anti-human TIMP-2 polyclonal antibody (gift of Dr. R. A. Hynes, Harvard Medical School) for 1 h at 37°C and washed with TBS washing buffer for 30 min. The secondary antibody, goat anti-rabbit IgG coupled to peroxidase, was diluted 1:4000 and incubated with the membrane for 30 min. After washing with TBS washing buffer for 30 min, the membrane was developed by the enhanced chemiluminescence method (ECL, Amer- sham Life Science, Inc.).
mechanical strains on intact FN can suppress cytokine-induced MMP-1 secretion raised the hypothesis that mechanical strains suppress MMP-1 secretion through non-RGD domains of FN. To explore this hypothesis, we plated VSMCs on 2 μg/ml 120FN, a concentration sufficient to support equivalent amounts of adhesion of cells as intact FN coating of a membrane. In contrast to previous reports on rabbit synovial fibroblasts, plating VSMCs on 120FN did not induce MMP-1, nor did mechanical strain induce MMP-1 expression by VSMCs on 120FN. PDGF induced MMP-1 in VSMCs plated on 120FN (5.2 ± 1.9-fold, n = 3), and 1 and 4% cyclic mechanical strains suppressed PDGF-induced MMP-1 by 37 ± 15% and 50 ± 23%, respectively (Fig. 1B). This experiment indicated that strain did not inhibit MMP-1 synthesis through adhesion to the connecting segment-1 region of FN.

In addition, we studied VSMCs plated on ovalbumin-coupled RGD peptide (2 μg/ml). MMP-1 expression by VSMCs on RGD peptides was similar to that by VSMCs on intact FN or 120FN (7.0 ± 3.3-fold, n = 3). Similar to the effects of FN and 120FN, PDGF induced MMP-1 expression on RGD peptides, but 1 and 4% mechanical strains also suppressed PDGF-induced MMP-1 by 52 ± 28% and 72 ± 28% of PDGF-induced MMP-1 synthesis, respectively (Fig. 1C). These data further support the finding that suppression of MMP-1 by small mechanical strains does not require the connecting segment-1 domain of FN and that mechanical strain through the RGD domain is sufficient for this effect.

Effect of Strain on MMP-2 and MMP-9—Although many MMPs have similar promoter elements, expression of the gelatinases, particularly MMP-2, may be independent of MMP-1 expression (25). Therefore, we evaluated the expression of MMP-2 (72-kDa gelatinase) and MMP-9 (92-kDa gelatinase) by VSMCs in response to mechanical stimuli and PDGF treatment. Neither mechanical strain nor PDGF treatment changed constitutive MMP-2 expression (Fig. 2A). VSMCs plated on 120FN or ovalbumin-coupled RGD peptide also expressed similar levels of MMP-2 regardless of PDGF treatment or mechanical strain (Fig. 2B and C). We also found MMP-9 activity in VSMCs plated on FN, but the activity was very weak compared with MMP-2 activity. Neither PDGF treatment nor mechanical strain increased MMP-9 expression (Fig. 2A). On 120FN or ovalbumin-coupled RGD peptides, MMP-9 induction was not observed compared with intact FN, and neither PDGF treatment nor mechanical strain increased MMP-9 expression (Fig. 2, B and C). These results eliminated the possibility that all MMPs were down-regulated by mechanical strain.

Effect of Strain on TIMP-1 and TIMP-2—We then explored the effects of mechanical strain on synthesis of TIMP-1 and TIMP-2, endogenous inhibitors of metalloproteinases. On FN, TIMP-1 expression was constitutive, whereas PDGF induced TIMP-1 expression by 1.9 ± 0.4-fold (n = 3). Similar to the effect of mechanical strain on MMP-1 synthesis, strains of 1 and 4% modestly suppressed PDGF-induced TIMP-1 expression by 27 ± 7% and 48 ± 8%, respectively (Fig. 3A). When VSMCs were plated on 120FN, TIMP-1 expression was similar to that of VSMCs plated on intact FN, and PDGF also induced TIMP-1 expression by 3.6 ± 2.2-fold (n = 3). On 120FN, mechanical strain alone did not affect TIMP-1 expression, but 1 and 4% strains suppressed PDGF-induced TIMP-1 expression by 23 ± 8% and 61 ± 53%, respectively (Fig. 3B). When VSMCs were cultured on ovalbumin coupled to RGD peptides, TIMP-1 expression was not induced, but PDGF induced TIMP-1 expression by 1.7 ± 0.1-fold. On RGD peptides, 1 and 4% mechanical strains suppressed PDGF-induced TIMP-1 expression by 31 ± 11% and 41 ± 35%, respectively (Fig. 3C). These results showed that the effect of strain on TIMP-1 regulation by VSMCs plated on intact FN, 120FN, or RGD peptides was similar to the effect of strain on MMP-1 synthesis.

In contrast to the effects of mechanical strain on MMP-1 and TIMP-1 expression, the expression of TIMP-2, the specific inhibitor of MMP-2, by VSMCs cultured on FN was not changed by PDGF treatment or mechanical strain (Fig. 4A). When VSMCs were cultured on 120FN or ovalbumin-coupled RGD
expression is specific to induction by PDGF, we treated VSMCs (26). To investigate if the effect of strain on MMP-1
expression induced by IL-1α or TNF-α—IL-1α and
TNF-α are potent inducers of MMP-1 expression by human
VSMCs (26). To investigate if the effect of strain on MMP-1
expression is specific to induction by PDGF, we treated VSMCs
with IL-1α (10 ng/ml) or TNF-α (10 ng/ml) after 48 h of serum
depression. Cyclic mechanical strain suppressed MMP-1 syn-
thesis induced by TNF-α, but strain did not suppress IL-1α-
induced MMP-1 synthesis (Fig. 5).

**Strain and MMP-1 Regulation by Human VSMCs Cultured on Collagen—**Extracellular matrix components may influence
both MMP-1 expression and mechano-responsiveness (8, 27).
VSMCs were plated on 50 μg/ml collagen, a concentration
optimal for VSMC adherence to the silicone membrane. Similar
to the response of VSMC plated on fibronectin, strain did not
induce MMP-1 expression, but suppressed MMP-1 synthesis
by PDGF induction even at 1% amplitude (Fig. 6).

**Northern Analysis—**To explore potential mechanisms of reg-
ulation of MMP-1 and TIMP synthesis by mechanical strain,
VSMCs cultured on fibronectin were subjected to 4% strain in
the presence of PDGF after 2 days of serum deprivation. North-
er analysis for MMP-1 showed that 4% strain suppressed
steady-state MMP-1 mRNA levels, whereas TIMP-1 mRNA
levels were not appreciably changed (Fig. 7).

Mechanical strains can induce c-Fos, a component of the
AP-1 complex that can promote transcription of MMP-1. Phos-
phorylation of c-Jun by JNK (c-Jun NH2-terminal kinase) is a
signaling mechanism stimulated by physical stress (28) such as
UV irradiation, heat shock, and several cytokines (29–31);
increased JNK activity is another mechanism by which me-
chanical strain may stimulate AP-1 activity (32). To explore the
potential mechanism of MMP-1 suppression by strain, cultured
VSMCs on FN were subjected to 4% strain. Induction of c-fos
was observed after 30 min of strain (Fig. 8). In addition, JNK
activity was not significantly affected (data not shown). Mem-
ers of the Ets family of transcriptional activators also regulate
MMP-1 expression by interacting with the AP-1 complex (33–
35). Northern analysis of *ets-1* demonstrated that strain sup-
pressed *ets-1* expression by PDGF (Fig. 8). Therefore, the sup-
pression of MMP-1 by small strains may be related to suppres-
sion of *ets-1*, but not suppression of c-fos.

**DISCUSSION**

Previous studies have suggested that mechanical injury to
cells and cytoskeletal deformation stimulate MMP synthesis
and secretion. In addition, activation of components of the AP-1
complex, a potent positive regulator of MMP-1 transcription,
can occur with mechanical deformation of cells. Thus, we ini-
tially anticipated that small mechanical deformations of
VSMCs would induce MMP-1 synthesis. In addition, we hy-
pothesized that this induction would be independent of, or
synergistic with, biochemical stimulation of MMP-1 syn-
thesis, such as by PDGF. Surprisingly, we found that small me-
chanical deformations do not induce MMP-1 synthesis, but poten-
tially suppress MMP-1 synthesis by PDGF. These findings indicate
that small mechanical deformations may be a powerful influ-
ence on cellular control of extracellular matrix degradation.
Huhtala et al. (17) and Werb et al. (36) showed that intact
fibronectin does not induce MMP-1, but the chymotryptic frag-
Strain Suppresses MMP-1 Expression

Table 1: Strain (%) on Collagen

| Strain (%) | 0   | 1   | 4   |
|------------|-----|-----|-----|
| PDGF       | +   | +   | +   |
| MMP-1      | +   | +   | +   |
| TIMP-1     | +   | +   | +   |
| 18S        |     |     |     |

Fig. 6. Effect of strain on MMP-1 synthesis by VSMCs cultured on collagen. VSMCs were cultured on 50 μg/ml collagen in DMEM containing 10% FCS for 24 h. After serum deprivation for 48 h, cells were subjected to 0, 1, and 4% cyclic mechanical strains (1 Hz) for 24 h with (+) or without (-) PDGF-BB (5 ng/ml), and media were analyzed by Western analysis with anti-human MMP-1 polyclonal antibody.

Fig. 7. Effect of strain on MMP-1 and TIMP-1 mRNA synthesis. VSMCs were cultured on intact FN (2 μg/ml) in DMEM containing 10% FCS for 24 h. After serum deprivation for 48 h, 0 and 4% cyclic mechanical strains were imposed for 0, 1, 3, 6, and 12 h with (+) PDGF-BB (5 ng/ml) treatment. Total RNA was isolated at each time point, and MMP-1 and TIMP-1 were analyzed by Northern analysis. The ethidium bromide-stained 18 S ribosomal subunit is also shown.

Fig. 8. Effect of strain on the expression of ets-1 and c-fos. VSMCs were cultured on intact FN (2 μg/ml) in DMEM containing 10% FCS for 24 h. After serum deprivation for 48 h, 0 and 4% cyclic mechanical strains were imposed for 0, 0.5, 1, 6, 12, and 24 h with (+) PDGF-BB treatment. Total RNA was isolated at each time point, and ets-1 and c-fos were analyzed by Northern analysis. The ethidium bromide-stained 18 S ribosomal subunit is also shown.

ment 120FN containing the RGD domain induces MMP-1 synthesis by rabbit synovial fibroblasts. In addition, the ability of non-RGD domains of fibronectin to suppress MMP-1 synthesis appears to be mediated by interactions with αβ-integrins rather than the RGD-binding classical fibronectin receptor, α5β1. In contrast, in these experiments, when human VSMCs were plated on fibronectin, 120FN, or RGD peptides, MMP-1 was not induced. The difference between these results may be due to differences between rabbit synovial fibroblasts and human vascular smooth muscle cells. For example, human VSMCs have very little α5β1 by immunoprecipitation (37), so perhaps non-RGD domains of fibronectin cannot regulate MMP-1 synthesis by VSMCs. The similarity of the strain effect on MMP-1 regulation by VSMCs on collagen implies that strain itself might be a powerful regulator of MMP-1 synthesis regardless of the type of extracellular matrix component. The matrix, integrins, plasma membrane, cytoskeleton, and nucleus are interconnected, and mechanical deformation of any cellular component may lead to a change in shape of another cellular component (38–41). Therefore, it is possible that integrins provide anchors to link a cytoskeleton or nuclear mechanotransduction mechanism to the extracellular space. Studies by Wilson et al. (8) suggest that αβ-integrins may regulate mechanotransduction, even when cells are adherent to matrices other than fibronectin or vitronectin. Further studies will be necessary to determine if mechanical strain through specific α- or β-integrin subunits can regulate MMP synthesis.

One of the advantages of the mechanical strain device used in this experiment is that strains are precise and highly uniform. In many other types of cell-stretching devices, regions of the cell culture substrate have very high strains compared with the mean strain. For example, in one commonly used device, the strain can range from 0 to 33% depending on the location and orientation of the cell (21). We have previously demonstrated that cellular injury and fibroblast growth factor-2 release occur only when VSMCs are exposed to membrane strains higher than 10% (22). Imposing small strains with this cellular deformation device prevents confusing paracrine effects (such as fibroblast growth factor-2 release) from non-uniform strain profiles. In addition, the precise strains imposed in these experiments eliminate the possibility that suppression of MMP-1 by strains as small as 1% was due to cellular injury. Furthermore, the experiments demonstrating that MMP-2 and TIMP-2 syntheses are not suppressed by small strains indicate that the suppression of MMP-1 is selective.

These experiments explored some potential mechanisms of the suppression of MMP-1 synthesis by strain. Strain augmented c-fos expression in the presence of PDGF, but suppressed ets-1 expression. However, there are at least 30 members of the Ets family, and mechanisms other than ets-1 down-regulation could play a major role. Westermarck et al. (42) reported that PU.1, a member of the Ets family, can suppress MMP-1 synthesis. In our studies, however, we have not found PU.1 expression by VSMCs. Our studies suggest that direct effects of the PDGF receptor are not primarily responsible for the effects of strain, as strain could also suppress MMP-1 induction by TNF-α. We have evaluated cytoskeletal changes under these conditions using rhodamine-phalloidin staining, which demonstrated no clear effect of strains on the actin cytoskeleton (data not shown); however, we cannot exclude important cytoskeletal changes as a mechanism for mechanotransduction under these conditions.

Previous reports have described mechanically mediated synthesis and release of PDGF from rat VSMCs subjected to mechanical deformation (7). In pilot studies, we have not observed induction of the PDGF-A or PDGF-B genes in human VSMCs exposed to the small mechanical deformations used in the present study. It is possible that species-specific responses or differences in the mechanical strain devices could explain these differences. Furthermore, mechanically mediated release of PDGF would not explain why small strains can suppress the induction of MMP-1 by exogenous PDGF.

2 J.-H. Yang, W. H. Briggs, P. Libby, and R. T. Lee, unpublished observations.
In vivo, MMPs are frequently found in tissues undergoing repair or remodeling, such as high stress locations of the human atherosclerotic lesion (18, 19, 43). In these circumstances, cytokines and growth factors are generally abundant and regulate the repair process. However, cells in a repairing tissue must also produce a new tissue that can withstand mechanical forces imposed on that tissue, such as the constant tension of forces imposed on that tissue, such as the constant tension of forces imposed on that tissue, such as the constant tension of forces imposed on that tissue, such as the constant tension of forces imposed on that tissue, such as the constant tension of forces imposed on that tissue, such as the constant tension of forces imposed on that tissue, such as the constant tension of forces imposed on that tissue, such as the constant tension of forces imposed on that tissue, such as the constant tension of forces imposed on that tissue, such as the constant tension of forces imposed on that tissue, such as the constant tension of forces imposed on that tissue, such as the constant tension of forces imposed on that tissue, such as the constant tension of forces imposed on that tissue, such as the constant tension of forces imposed on that tissue, such as the constant tension of forces imposed on that tissue, such as the constant tension of forces imposed on that tissue, such as the constant tension of forces imposed on that tissue, such as the constant tension of forces imposed on that tissue, such as the constan...