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Mechanical Strain Induces a Persistent Upregulation of Syndecan-1 Expression in Smooth Muscle Cells

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Syndecan-1 belongs to a family of transmembrane proteoglycans, acts as a coreceptor for growth factor binding, as well as cell–matrix and cell–cell interactions, and is induced in smooth muscle cells (SMCs) following balloon catheter injury. In this report, we investigated syndecan-1 expression in SMCs in response to several distinct biomechanical force profiles and the related syndecan shedding response. Syndecan-1 mRNA expression increased in response to 5% and 10% cyclic strain (24 h: 206 ± 40% and 278 ± 33%, respectively, $P < 0.05$) when compared to unstrained controls. When subjected to 10% cyclic strain for periods of up to 48 h, syndecan-1 mRNA levels remained elevated at 294 ± 31%. Notably, the SMC mechanosensor mechanism remained responsive after an initial 24 h "preconditioning" period, as evident by a fivefold increase in syndecan-1 gene expression following a change in cyclic stress from 10% to 20% (48 h: 516 ± 55%, $P < 0.05$). Of note, similar behavior was not observed in an analysis of syndecan-2 mRNA levels. Commensurate with mRNA responses, mechanical stress induced an increase in cell-associated syndecan-1 protein levels with an associated increase in protein shedding. Given the varied functions of syndecan-1, stress-induced effects on SMC syndecan-1 expression and shedding may represent an additional component of the pro-inflammatory, growth-stimulating pathways that are activated in response to changes in the mechanical microenvironment of the vascular wall. Syndecan-1 expression is uniquely influenced by changes in the phase and magnitude of the local stress field.

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Syndecan-1 is a member of a family of cell surface heparan sulfate proteoglycans (HSPG) and is capable of modulating cell behavior through its capacity to act as cell surface co-receptors for a variety of heparin binding proteins. Recent investigations have emphasized that accelerated syndecan shedding may provide an important mechanism for regulating local host responses to tissue injury (Park et al., 2000b). Indeed, agents that increase syndecan shedding, including a variety of proteases and growth factors, are released during acute wound repair (Subramanian et al., 1997; Kainulainen et al., 1998; Fitzgerald et al., 2000; Park et al., 2000a). Moreover, several reports have noted that an absolute reduction in these cell surface receptors, as a consequence of shedding, may alter cell responses to both soluble and insoluble heparin binding proteins.

Although shedding may alter the cell surface concentration of syndecans, it is noteworthy that shed HSPGs may exert a direct biological effect through the presence of heparan sulfate (HS) glycosaminoglycans that remain attached to the shed core protein. Through retention of ligand binding activity, shed syndecans are capable of sequestering heparin binding growth factors, such as FGF-2, protecting these factors from heat, pH, and protease-related degradation mechanisms present in the extracellular matrix. After heparanase-induced cleavage of HS chains on the shed syndecan core protein, heparin binding proteins are then able to interact with cell bound receptors (Kato et al., 1998). Alternatively, shed syndecans may potentially act as dominant negative modulators by competing with cell surface receptors for the same ligand. All told, considerable evidence now supports the notion that shed syndecan ectodomains likely have important physiologic roles in morphogenesis, tissue repair, and host defense. In this regard, Wang et al. (1997) and Nikkari et al. (1994) have observed persistent upregulated expression of syndecan-1 mRNA in arterial neointima after balloon injury in rabbit and rat models, respectively. Although a direct causal relationship has yet to be proven, we have postulated that locally accelerated syndecan shedding may lead to maladaptive responses that play a critical role in vascular lesion formation.

The restenosis response that often follows balloon angioplasty with or without stent placement may be due, in part, to extreme vessel wall stress that is associated with this mechanical intervention (Moore and Berry, 2002). Although balloon angioplasty represents an acute change in local stress, chronic changes in the mechanical microenvironment may also be responsible for the development of neointimal hyperplasia at vascular anastomoses (Perktold et al., 2002). Though not specifically modeled by our experiments using arterial SMCs, the mechanical microenvironment likely also plays an important role during the arterialization of a vein graft after its translocation from a low to a high pressure environment (Shi et al., 2001). Moreover, systemic arterial hypertension is an independent and potent risk factor for the development of atherosclerosis, and a direct correlation has been noted

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between regions of increased wall tension and sites that are predisposed to the formation of atherosclerotic lesions (Thubrikar et al., 1990; Thubrikar and Robicsek, 1995). Recent investigations have also emphasized that the magnitude of the phasic changes in wall stress may be an important stimulus for atherosclerosis. For example, in a study of 19,083 men, pulse pressure was the strongest predictor of cardiovascular mortality (Benetos et al., 1997).

In this report, we investigated syndecan-1 expression in response to several distinct force profiles that are representative of varied mechanical microenvironments that may be experienced by vascular SMCs in vivo. In selective experiments, we compared these responses to syndecan-2 and demonstrate that syndecan-1 expression is uniquely influenced by changes in the phase and magnitude of the local stress field. Moreover, we determined that cyclic strain causes a dramatic increase in both the expression of cell-associated syndecan-1, as well as shedding of this HSPG.

Materials and Methods

Cell culture

Clonetics normal human aortic smooth muscle cells (SMC), culture medium, and supplements were obtained from Cambrex Bio Science (Walkersville, MD). Multiple different batches of SMCs were used and data averaged accordingly. Smooth muscle cell growth medium-2 (SmGM-2) was prepared according to the supplier's recommendations. Cells were maintained in tissue culture-treated petri dishes at 37°C, 5% CO_2, and humidified atmosphere. Growth medium was changed every 2 days, and the cells were passaged 1:4 when the dishes were 80% confluent, using 0.05% Trypsin/0.53 mM EDTA (Invitrogen Life Technologies, Carlsbad, CA). Experiments were performed on cells harvested between passages 6 and 10 using SmGM quiescence medium (0.5% FBS) or SmGM complete medium (5% FBS), as detailed below.

Mechanical strain application

While the in vivo complexity of both the biomechanical and associated biochemical events cannot be fully captured in a cell culture system, in vitro model assays can replicate force profiles that are representative of those experienced by vascular SMCs in vivo. In general terms, changes in the biomechanical microenvironment relevant to the development of lesions within the vascular wall may be considered to fall within three distinct force profiles. First, balloon angioplasty induces an acute increase in wall tension that on a cellular level may be recapitulated in vitro by a single mechanical stretch of cells cultured on an elastomeric substrate (Zarins et al., 1982; Waller, 1989; Honye et al., 1992; Lyon and Gallagher, 1998). Second, states that transiently increase blood pressure may lead to an acute increase in cyclic wall tension. As a third regime, sustained or poorly controlled hypertension may be represented biomechanically by a persistent increase in cyclic tension. As illustrated in Figure 1, SMCs were subjected to one of three regimens of cyclic or static strain. Of note, current evidence suggests that SMCs in the medial layer of the vascular wall are subjected in vivo to homogeneous and biaxially strains. Moreover, strain amplitudes of 10% in vitro are physiologically relevant to strains present in large arteries, including the aorta, carotid, femoral, and brachial arteries in vivo (Wijnen et al., 1991; Chen et al., 2003).

Strain dishes were assembled by fitting the bases of bottomless, custom-made plastic petri dishes with a silicone membrane (0.005″ thickness; 40 Durometer; Specialty Manufacturing, Saginaw, MI). In all experiments, 5 μg/ml of human plasma fibronectin (FN; Sigma, St. Louis, MO) in PBS was preadsorbed to the growth area for at least 8 h at 4°C, serving as a substrate for cell attachment. The cells were then seeded onto the membranes, cultured to 80% confluence in SmGM-2, and then growth-arrested for 24 h in quiescence medium for subsequent RNA analysis. Protein analysis was performed in 5% FBS to overcome low basal expression in quiescence medium (Cizmeci-Smith and Carey, 1997). Stretch-induced protein levels were compared to unstretched control cells, seeded at equivalent densities in strain dishes. Depending on the experimental conditions, defined static or cyclic tension was applied to the cells using a StrainMaster apparatus (Z-Development, Inc., Cambridge, MA) that allowed control over the frequency and amplitude of the radial and circumferential strains of the membrane and enabled the application of homogeneous and biaxially uniform strains over the entire growth area of the membrane. The radial and circumferential strain profiles provided by this device have been detailed elsewhere (Hung and Williams, 1994; Schaffer et al., 1994; Cheng et al., 1997). For a given protocol, cells were either cyclically strained at 1 Hz or held static at predefined strain amplitudes and harvested at various time points. In selected experiments, cells were preconditioned at 10% cyclic strain for 24 h, followed by 20% strain thereafter. A two color Live/Dead cell assay confirmed that cells

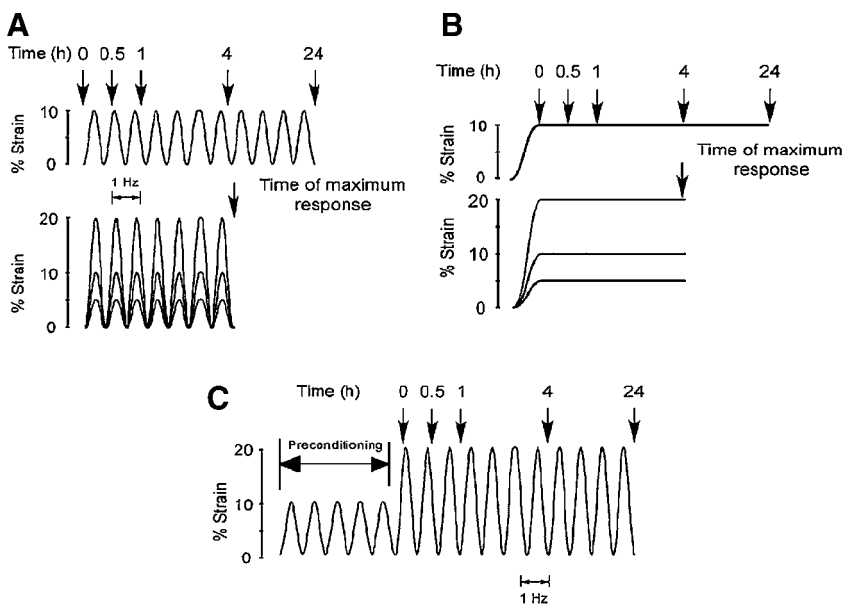

Fig. 1. Protocols for the application of mechanical strain. SMCs were subjected to 1 Hz cyclic tension for various time periods and strain magnitudes (A). Alternatively, cells were subjected to static tension (B). The effect of preconditioning was examined by cyclically stretching the cells at 10% strain for 24 h, followed by 20% cyclic strain (C). Arrows indicate sampling points, in which cells were harvested for analysis.

remained viable under all mechanical conditions imposed in these studies (see Fig. S1, Supporting Data).

Human aortic SMC RNA analysis

Real time quantitative PCR was used to determine syndecan gene expression in human aortic SMCs. Briefly, total RNA was harvested using TRIzol reagent (Gibco BRL, InVitrogen, Carlsbad, CA) in which RNA was extracted with chloroform and isopropanol, rinsed with 75% ethanol, and re-suspended in RNase-free water. The absence of significant DNA or protein contamination was verified by UV absorbance ratio (OD_{260}/OD_{280}). cDNA was generated using SuperScript III reverse transcriptase (Invitrogen) using 5 μg of RNA. Real time PCR was performed using the TaqMan primer and probes for syndecan-1 (Hs00174579_m1), syndecan-2 (Hs00299807_m1), and 18S rRNA (Hs9999901_s1). Amplification and detection was performed on an ABI Prism 7000 Sequence Detection System using AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA) in a 5′ nuclease reaction. Data generated from syndecan gene amplification was normalized to the simultaneously amplified endogenous 18S rRNA controls. Gene expression in mechanically strained cells was represented relative to that measured in unstrained controls.

Syndecan-1 immunoblot analysis

Specificity and quantitation of the immunoblot assay for syndecan-1 analysis has been previously described in the literature (Elenius et al., 1992; Sneed et al., 1994). SMCs were seeded at equivalent densities in custom strain dishes as described above. Syndecan-1 protein expression was analyzed relative to unstrained control cells that were manipulated exactly as experimental cells, without the application of strain. Cell-associated and shed syndecan-1 was analyzed at 0, 4, and 24 h time points, the 0 h time point was defined by the addition of fresh culture medium to strain dishes. Shed syndecan-1 was measured in conditioned culture medium at indicated time points, conditioned medium was collected, and passed through a 0.2 μm membrane filter (Millipore, Billerica, MA) to remove detached cells. Media from one culture dish was concentrated to 400 μl using a 10,000 MWCO Amicon Ultra-15 centrifugal filter and utilized for subsequent shed protein analysis. Cell surface syndecan-1 was measured in the trypsinized cell fraction, as described previously (Subramanian et al., 1997). Briefly, following removal of media, cells were washed once with TBS, 0.5 mM EDTA at 4°C, and incubated for 15 min at 4°C with 10 μg/ml TPCK-treated trypsin. Following the 15 min incubation period, trypsin was inactivated with 100 μg/ml of soybean trypsin inhibitor and detached cells were removed by centrifugation. Prior to immunoblot analysis, trypsinized fractions were concentrated using a 10,000 MWCO Amicon Ultra-15 centrifugal filter. Immunoblot analysis was performed using cationic polyvinylidene difloride-based membranes (Immobilon-N, Millipore) under mild vacuum in a Bio-Dot SF slot blot apparatus (Bio-Rad, Hercules, CA). Shed and cell-associated samples were acidified in 50 mM NaOAc (pH 4.5), 150 mM NaCl, 0.1% Tween 20 to increase specificity of proteoglycan blotting (Park et al., 2000a). Blotted membranes were blocked for 1 h at room temperature in 5% nonfat dry milk in 0.04% Tween-TBS pH 7.4. The immunoblot was labeled overnight at 4°C with anti-syndecan-1 mAb (human CD138 biotinylated, Cell Sciences, Canton, MA) diluted 1:300 in 1% nonfat dry milk in 0.04% Tween-TBS pH 7.4. The membrane was washed in 0.04% Tween-TBS pH 7.4, followed by incubation with streptavidin-HRP, diluted 1:3,000 in blocking buffer for 1 h. The immunoblot was developed using enhanced chemiluminescence and labeled protein detected using BioMax MR autoradiography film. Levels of protein were quantified by densitometry using ONE-Dscan image analysis software (Scanalytics, BD Biosciences, Rockville, MD). Positive and negative control blots confirmed that the antibody did not crossreact or bind nonspecifically (see Fig. S2). All assays were performed in triplicate.

Statistics and data analysis

In order to analyze changes in syndecan-1 and syndecan-2 mRNA expression, quantities at given experimental time points were divided by the quantities of the 0 h time points (unstrained controls) for each experiment. Consequently, the 0 h time points always had the value of 1.0 (100% of control), and thus were not presented graphically in the figures. For the experimental data, expression levels presented as greater than 100% of control represent increases, while those presented as less than 100% of control represent decreases. Values are reported as mean ± standard deviation of at least triplicate experiments. In each experiment, samples were collected from four separate dishes containing SMCs. Statistical analysis was performed using the SigmaStat 3.0 software package and where applicable, logarithmic transformation of the data was performed. ANOVA was employed, using the Holm-Sidak or Tukey tests for multiple pairwise comparisons and the Kruskal–Wallis nonparametric test used to assess three or more unpaired groups when normality assumptions did not apply. Interaction effects between two independent variables were examined by two-way ANOVA. A P-value <0.05 was considered statistically significant for all tests.

Results

Acute changes in strain induce syndecan-1 and -2 expression in vascular SMCs

Syndecan-1 and -2 mRNA expression was initially investigated in response to static strain (Fig. 2). When subjected to either 5% or 10% static strain syndecan-1 did not exhibit a significant change in expression over a 24-h observation period. In contrast, syndecan-2 displayed a significant decrease in expression. Following 10% static strain, syndecan-2 gene expression fell to 53 ± 12% of the unstrained control at 24 h ($P < 0.05$). Statistical analysis did not reveal a significant dependence of gene expression on the magnitude of *static* strain at any of the time points under investigation.

Given the results from the static strain experiments, we sought to evaluate whether the pattern of syndecan expression was purely a stretch-related event, or whether the periodic nature of cyclic strain was important regulator of expression (Fig. 3). Both syndecan-1 and syndecan-2 responded to 5% cyclic strain with steady increases in gene expression. Syndecan-1 displayed significant differences between 1 and 24 h and between 4 and 24 h, increasing from 93 ± 6% of unstrained controls at 1 h to 131 ± 14% at 4 h to 206 ± 40% at 24 h. Syndecan-2 displayed significant increases between all three time points, increasing from 67 ± 7% of unstrained controls at 1 h to 117 ± 8% at 4 h, to 186 ± 17% at 24 h. In response to 10% cyclic strain, syndecan-1 mRNA expression increased significantly from 107 ± 10% of unstrained controls at 1 h to 278 ± 33% at 24 h. Similarly, syndecan-2 significantly increased from 97 ± 14% of unstrained controls at 1 h to 204 ± 17% at 24 h. Of note, unlike syndecan-2, a statistically significant dependence of gene expression on strain magnitude was displayed by syndecan-1. After a 24-h period of cyclic strain, SMCs exhibited an increase in syndecan-1 mRNA synthesis of two- to threefold upon an increase in cyclic strain amplitude from 5% to 10% strain, respectively ($P < 0.05$).

In order to assess the syndecan gene regulation under more sustained periods of cyclic strain, SMCs were subjected to 10% cyclic strain for periods of up to 48 h (Fig. 4). Both syndecan-1 and syndecan-2 mRNA levels remained elevated at 294 ± 31% and 222 ± 43%, respectively, compared to unstrained controls ($P < 0.05$).

Syndecan-1 expression remains responsive to changes in cyclic stress in mechanically preconditioned smooth muscles cells

SMCs in vivo exist in a mechanically dynamic oscillatory environment. Thus, we sought to determine whether expression of syndecan-1 or -2 is influenced by a change in mechanical microenvironment after a 24-h period of 10% cyclic strain. Specifically, cells were subjected to a 10% increase in strain amplitude or, in other words, a total cyclic strain amplitude of 20% for an additional 24-h interval (Fig. 5). Syndecan-1 mRNA levels increased significantly from 278 ± 18% of unstrained controls at end of the preconditioning period to 516 ± 55% after an additional 24 h of the 20% cyclic strain. During this same time period, syndecan-1 expression for

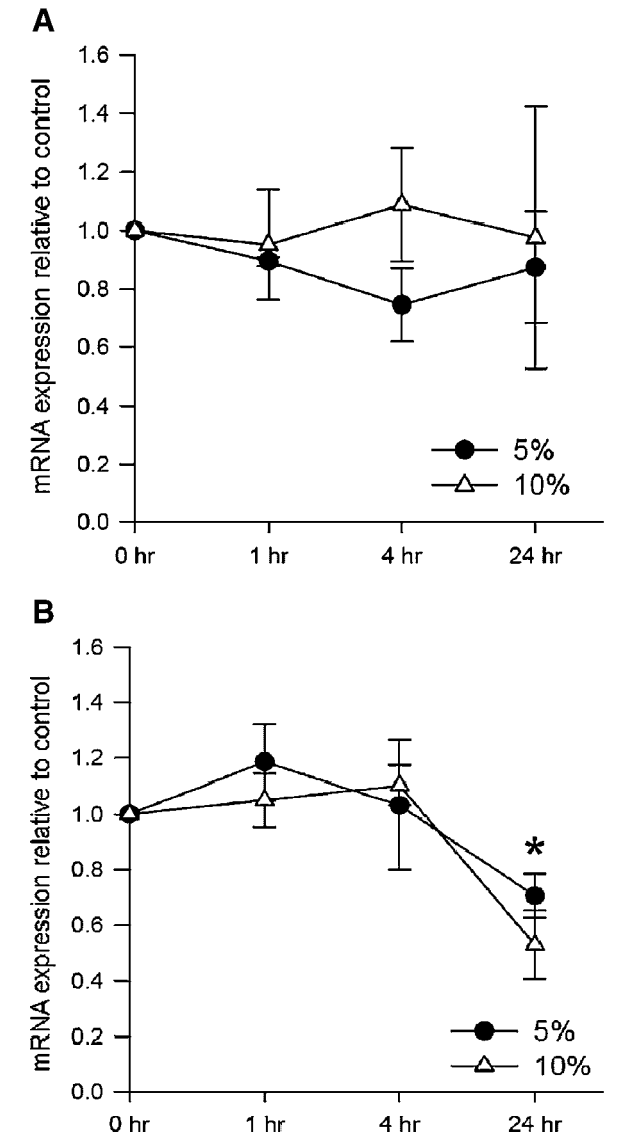

Fig. 2. Effect of static strain on syndecan-1 (A) and syndecan-2 (B) gene expression. Human aortic SMCs were cultured on elastic membranes, which were subjected to a single static stretch at 5% or 10% strain. All results were internally normalized to 18S rRNA and levels presented relative to unstrained controls. The data represent the mean and standard deviation of triplicate experiments, and statistical analysis was performed using ANOVA, employing Holm-Sidak's method for multiple pairwise comparisons ($^{*}P < 0.05$).

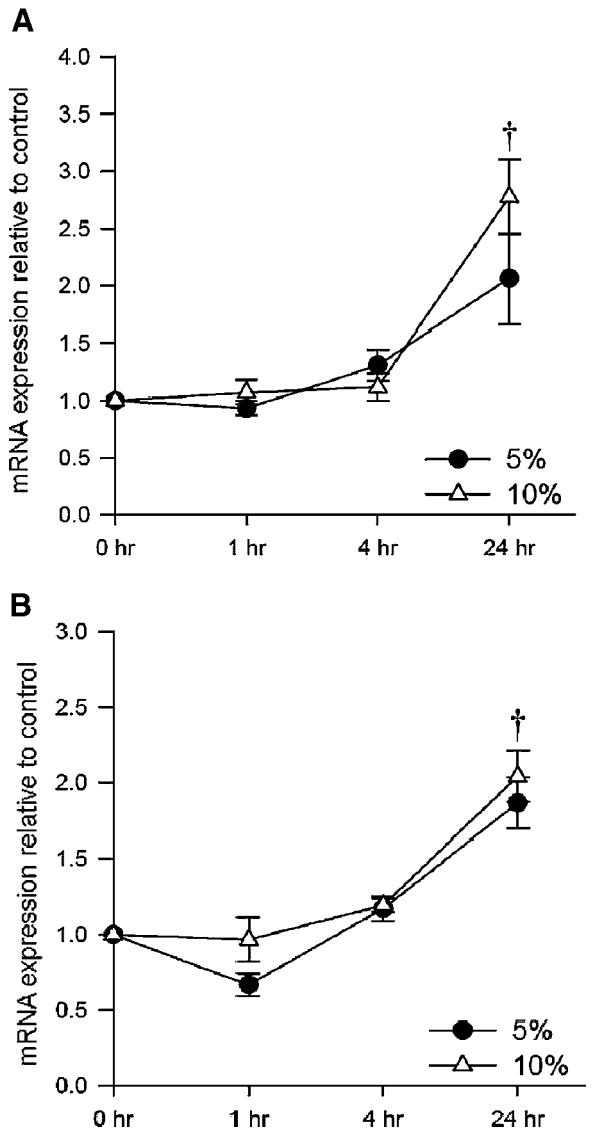

Fig. 3. Effect of 5% and 10% cyclic strain on syndecan-1 (A) and syndecan-2 (B) gene expression. Human aortic SMCs were cultured on elastic membranes, which were stretched at 1 Hz. All results were internally normalized to 18S rRNA and levels presented relative to unstrained controls. The data represent the mean and standard deviation of at least triplicate experiments. ANOVA was performed, employing Holm-Sidak's method for multiple pairwise comparisons ($^{\dagger}P < 0.05$).

SMCs maintained at 10% cyclic strain remained constant, ranging from 277 ± 9% of unstrained controls to 299 ± 33%. In contrast, syndecan-2 gene expression was not significantly affected by a 10% step increase in strain magnitude. Whether strain was maintained at 10% or increased to a total of 20% cyclic strain, relatively limited changes in syndecan-2 mRNA levels were observed. Specifically, syndecan-2 expression in cells maintained at 10% cyclic strain ranged from 204 ± 17% after the 24 h preconditioning period to a maximum of 283 ± 17% observed 4 h later (28 h). In cells subjected to an additional step increase of 10% cyclic strain, gene expression increased to only 254 ± 28% of unstrained controls 4 h after initiation of 20% cyclic strain. These changes in syndecan-2 expression were not statistically significant. Thus, in contrast to syndecan-2, syndecan-1 displayed a capacity for further augmentation in gene expression in response to a further increase in cyclic strain.

Cyclic strain regulates shed and cell-associated syndecan-1 protein

Given the significant responsiveness of SMC syndecan-1 to changes in mechanical microenvironment, the relationship of the observed changes in syndecan-1 mRNA levels to protein expression was investigated. Since syndecan-1 can be released from the cell membrane via ectodomain shedding,

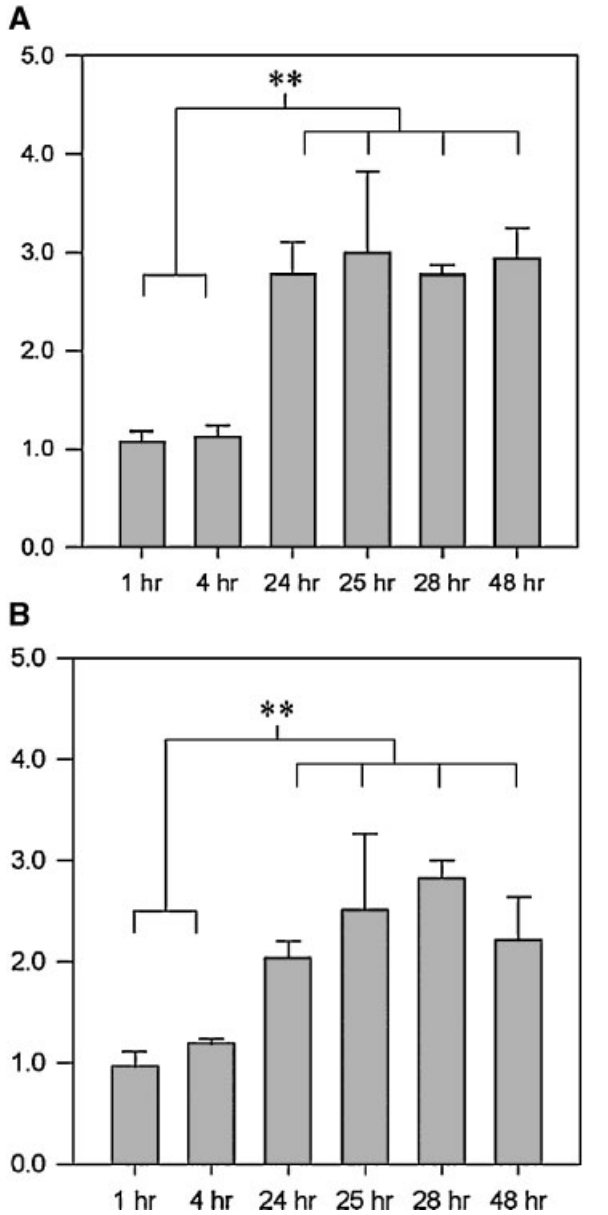

Fig. 4. Effect of 10% cyclic strain on syndecan-1 (A) and syndecan-2 (B) gene expression. Human aortic SMCs were cultured on elastic membranes which were stretched at 1 Hz for the indicated periods of time. The cells were then harvested, and RNA was isolated and analyzed using real time PCR. All results were internally normalized to 18S rRNA levels, and the syndecan levels are presented relative to unstrained controls. The data represent the mean and standard deviation of at least triplicate experiments, and when necessary, statistical analysis was performed on the data after logarithmic transformation. ANOVA was then performed, employing Holm-Sidak's method for multiple pairwise comparisons (indicates a *P*-value <0.05).**

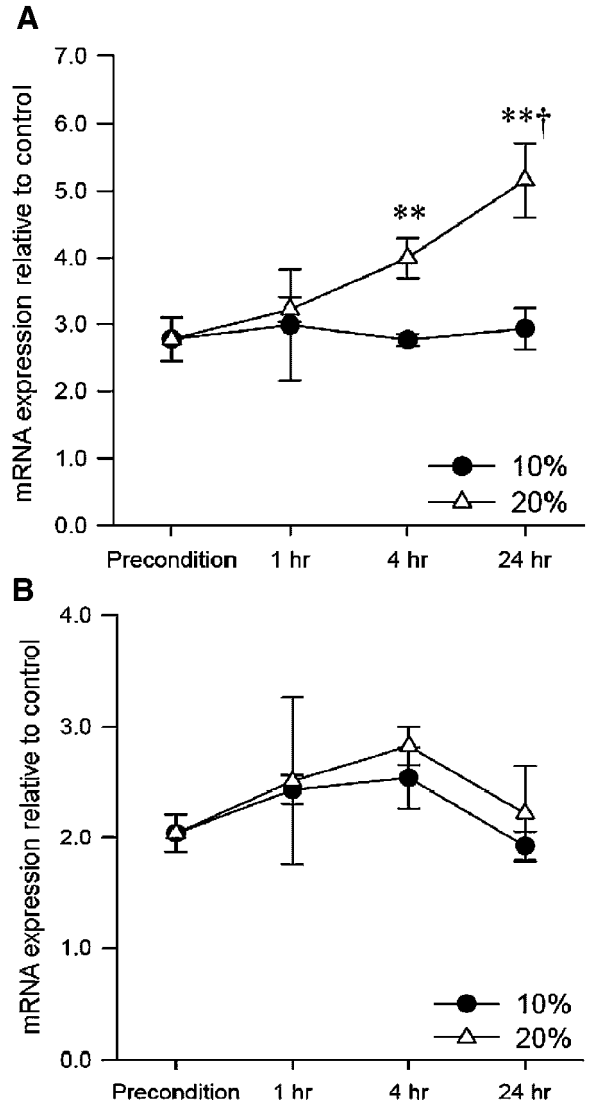

Fig. 5. Effect of preconditioning cyclic strain induced on syndecan-1 (A) and syndecan-2 (B) gene expression. Human aortic SMCs were cultured on elastic membranes, which were stretched at 1 Hz at 10% cyclic strain for 24 h, followed by 10% (●) or 20% (△) cyclic strain. All results were internally normalized to 18S rRNA and levels presented relative to unstrained controls. The data represent the mean and standard deviation of at least triplicate experiments. ($P < 0.05$ for comparisons with preconditioned cells. †$P < 0.05$ for comparisons between treatment groups at the indicated time point.)**

characterizing the effect of mechanical strain on syndecan-1 expression required an analysis of both cell-associated and shed protein (Fig. 6). Shed protein levels increased throughout a 24-h observation period and was significantly increased by the imposition of 10% cyclic strain. Likewise, consistent with mRNA data, 10% cyclic strain induced a significant increase in cell-associated syndecan-1 protein levels.

Discussion

Previous studies from our group suggest that changes in local mechanical stress within the vascular wall influences matrix-driven SMC adhesive and migratory behavior due, at least in part, to alterations in cell surface levels of syndecan-4 and by the shedding of these HSPGs into the pericellular environment (Li et al., 2002). For example, due to the involvement of syndecan-4 in the development of focal

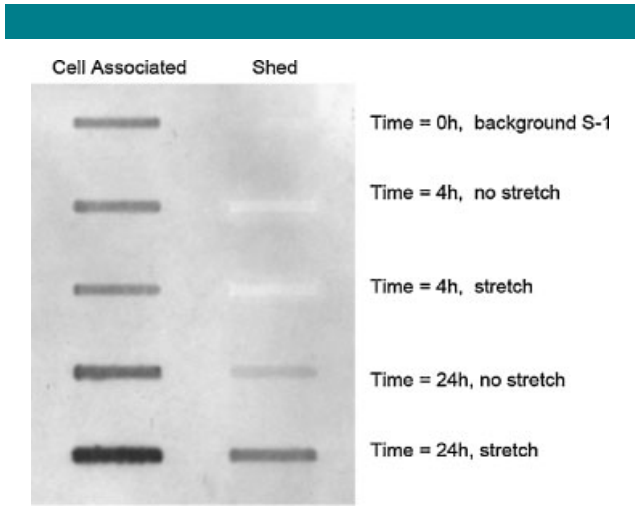

Fig. 6. Effect of 10% cyclic strain on syndecan-1 protein expression and shedding. Human aortic SMCs were cultured on elastic membranes, which were stretched at 1 Hz.

adhesions and stress fibers, loss of syndecan-4 may lead to a decrease in focal adhesions, with an increase in cell proliferation, migration, and secretion of ECM components. Likewise, the activity of heparin-binding growth factors, cytokines, proteases, and related ligands may be altered by their interactions with shed syndecans in a manner that promotes local tissue repair. We, and others, have noted that syndecans may promote local sequestration of growth factors and cytokines, thereby inducing cellular chemotaxis, which may be necessary for the effective resolution of tissue damage (Kato et al., 1998; Li et al., 2002).

Syndecan-1 displays both HS and chondroitin sulfate side chains and its expression is highly regulated in tissues. For example, syndecan-1 is expressed in the embryonic mesenchyme of the lung, kidney, and limb, but cannot be detected in differentiated sketetal or cardiac muscle (Saunders et al., 1989). The extracellular domain of syndecan-1 binds to bFGF and many ECM proteins, including fibrillar collagens (collagen type I, III, V), fibronectin, thrombospondin, and tenascin (Sun et al., 1989). Indeed, as receptor associated with cell adhesion and migration behavior, the intracellular domain of syndecan-1 is associated with actin of the cytoskeletal system (Carey et al., 1994) and syndecans released from the cell membrane appear to enhance SMC migration in vitro. Kiefer et al. (1990) have shown that syndecan-1 can bind to bFGF via its HS chains and Numa et al. (1995) demonstrated that over-expression of syndecan-1 in a renal epithelial cell line results in a higher cellular growth rate. HSPG genes, including syndecan-1, are activated in injured arteries and may play a role in regulating intimal thickening (Nikkari et al., 1994). For example, HSPG isolated from rat arterial wall suppresses the neointima formation when introduced into injured arteries (Bingley et al., 1997). The HS chains of the shed syndecan-1 ectodomains bind and regulate the activity of proteases, including neutrophil elastase and cathepsin G, and thereby may play an important role in the regulation of wound repair through interaction with growth factors, matrix proteins, or proteolytic enzymes (Park et al., 2000b). It is also significant that syndecan-2 interacts with matrix proteins such as laminin (Utani et al., 2001) and fibronectin (Kusano et al., 2000), and its cytoplasmic V region controls matrix assembly at the cell surface (Klass et al., 2000). Moreover, syndecan-2 can bind and modulate the signaling of TGF-β, as well as regulate the levels of TGF-β receptors (Chen et al., 2004; Fears et al., 2006).

In this report, we observed that syndecan-1 gene and protein expression in vascular SMCs is uniquely affected by distinct force profiles. For example, in response to static stress little alteration in syndecan-1 or syndecan-2 mRNA levels was initially observed, although a reduction in syndecan-2 expression below initial levels was noted at 24 h. In contrast, when SMCs were subjected to *cyclic* strain syndecan-1 gene expression displayed a monotonic response over a 24-h period. Although a similar response was observed for syndecan-2, the latter effect did not display a direct dependency on strain magnitude. Moreover, in contrast to syndecan-2, a *sustained increase* in gene expression in syndecan-1 was noted after an imposed increase in cyclic stress following an initial 24-h "preconditioning" period. While mechanical preconditioning has been most commonly applied to studies directed at elucidating reproducible mechanical responses of a material or tissue to an imposed load, preconditioning may also be useful in defining reproducible biological responses to a change in the mechanical environment (Birukov et al., 2003; Gonen-Wadmany et al., 2004). In this system, preconditioning lead to a fivefold increase in syndecan-1 at the end of the observation period. These data support the notion that, at least with respect to syndecan-1, the mechanism by which cells sense a mechanical stimulus can be reset once a new biomechanical environment is established. Notably, this response is quite unlike that observed with syndecan-4, which exhibits a bimodal response in a similar test protocol with a transient maximum 4 h after initiation 10% step increase in cyclic strain (Li et al., 2002). The kinetics of syndecan-1 protein expression was characterized by a steady increase in total cell syndecan-1 after the onset of an imposed cyclic stress, which paralleled an observed increase in shed syndecan-1. Thus, the increase in syndecan-1 gene expression appears to be a prerequisite response to an increase in total syndecan-1 protein expression, as well as an increase in shedding above initial constitutive levels. Significantly, prior studies from our group have demonstrated that alterations in the mechanical environment lead to only a transient increase in acute syndecan-4 shedding with a rapid return to basal levels of constitutive shedding (Li and Chaikof, 2002; Li et al., 2002). Syndecan-1 mRNA levels were persistently increased with establishment of a sustained elevated level of ectodomain shedding. Prior reports suggest that accelerated syndecan ectodomain shedding is a matrix metalloproteinase (MMP)-dependent process, in which different metalloproteinases regulate constitutive and accelerated shedding in a species-specific manner. Specifically, investigations in a mouse model suggest that signaling pathways converge to activate the tissue inhibitor of metalloproteinase (TIMP)-3-sensitive zinc metalloproteinase, MMP-7, while in human cells the membrane-type MMPs MT1-MMP and MT3-MMP appear to be involved (Fitzgerald et al., 2000; Endo et al., 2003). Of note, the proteolytic pathway for constitutive shedding appears to be distinct from that associated with accelerated syndecan shedding and, likewise, remains incompletely defined. Given that syndecan-1 is regulated in SMCs in response to diverse mechanical stimuli and the potential exists for a wide range of functional roles for both cell surface and shed syndecan-1, further studies are warranted to determine the specific shedding signaling pathway that are activated, as well as the physiologic significance of syndecan-1 in the evolution of vascular wall pathology in vivo.

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