AN ABSTRACT OF THE THESIS OF

Benjamin G. Steyer for the degree of Honors Baccalaureate of Science in Bioengineering presented on August 19, 2010. Title: The Role of Syndecan-1 in Arterial Mechanotransduction.

Abstract Approved: _____

Dr. Joseph McGuire

Hemodynamic forces regulate vascular homeostasis and arterial structure through mechanical stimuli by a process called mechanotransduction. Cellular response to mechanical forces involves coordination between cell surface receptors and intracellular signaling pathways. While previous work has focused on the role of integrin receptors in mechanotransduction, little is known about the role of cell surface proteoglycans in these processes. This work focuses specifically on the role of the cell surface proteoglycan syndecan-1 in vascular cell mechanotransduction. Mechanical forces were applied to rat aortae in an *ex vivo* culture system to examine the regulation of syndecan-1 expression. In addition vascular smooth muscle cells were isolated from syndecan-1 knockout mice and transfected with wild-type and mutated forms of syndecan-1 using lentiviral vectors. Wild-type, syndecan-1 knockout and transgenic human syndecan-1 mouse vascular smooth muscle cells were cultured in plates with collagen coated silicone membranes. An *in vitro* mechanical loading system was used to subject the cells to 1 Hz biaxial or uniaxial stretch at 10% strain at for up to two hours. We found that syndecan-1 knockout or deletion of the cytoplasmic region of syndecan-1 caused an increase in actin stress fibers and focal adhesion sites in response to mechanical strain. Further, knockout of syndecan-1 led to enhanced ERK phosphorylation in response to mechanical strain. These findings support that syndecan-1 is an important mediator of mechanotransduction in vascular cells.

Key Words: Syndecan-1, Mechanotransduction, Vascular Smooth Muscle Cells, Focal Adhesion

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# The Role of Syndecan-1in Arterial Mechanotransduction

by

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Benjamin G. Steyer, author

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# I. INTRODUCTION

## Mechanical stimuli regulate vascular adaptation

The development of closed vascular systems that enable fast and efficient transport of oxygen and nutrients marks a defining step in vertebrate evolution. The ability of these vascular systems to respond to environmental changes is also essential for maintenance of homeostatic conditions within the tissues of complex organisms [1]. It is widely accepted that the major effecter of this vascular tuning and regulation is mechanical stimuli experienced by the vascular tissue under biological conditions [2-3].

Vascular tissue is composed of two major tissue types, endothelial cells (EC) that line the interior lumen of the vessel, and vascular smooth muscle cells (VSMC) that provide structural support to the endothelial layer and give the vessel elastic properties. During normal circulatory processes, contractions of the heart drive volumes of blood through the proximal vasculature. This hemodynamic flow exerts cyclic stretch (strain) and shear stress on the vascular tissue. ECs are primarily exposed to shear stresses from blood flow whereas VSMCs are primarily influenced by cyclic stretch [3]. Endothelial response to sheer stress has been well characterized in the literature and was therefore not covered in this thesis. However, knowledge of VSMC response to cyclic stretching, while similar, remains limited and is the primary focus of this work.

Pulsatile blood flow causes arteries in close proximity to the heart expand at systole, the peak pressure during contraction of the left ventricle, and then deflate as pressure drops during diastole. The elastic nature of the vessels dampens the pulsatile pressure changes and as a result smoothes the periodic changes in velocity and pressure as blood moves

towards the delicate capillary beds. Studies have shown that cyclic stretching of VSMCs is essential for maintaining vascular tone and overall vessel function [4]. Acute changes is blood pressure are mediated by VSMCs through a mechanism called the myogenic effect, whereby elevated blood pressure triggers restriction of vessels to increase resistance to flow therefore slowing blood flow to capillary vessels [5]. If pressure remains high for long durations, as is the case with chronic hypertension, vascular cells undergo substantial remodeling as the tissue adapts to changes in hemodynamic force. These changes are often expressed in vascular wall thickening in response to increased stretch force, and EC realignment in response to changes in shear profiles [6].

**Vascular cell integration with the extracellular matrix**

Vascular tissue is functionally and structurally integrated with its surrounding extracellular matrix (ECM). Transmembrane adhesion receptor complexes tether ECM proteins to the cytoskeletal components within individual cells [7-12]. These physical connections enable the transmission of external mechanical stimuli to intracellular domains where they induce signaling process that alter cellular proliferation, apoptosis (programmed cell death), movement, and synthesis or degradation of ECM [9,11]. The overarching term referring to the process where mechanical stimuli are converted into signaling processes is mechanotransduction.

Changes in mechanical forces outside homeostatic ranges for prolonged periods can lead to substantial alteration of vascular tissue morphology. Vascular remodeling is implicated in chronic atherosclerotic conditions that can lead to heart attack and stroke [10,13]. These events together are responsible for about half of the total deaths reported in developed countries [1]. Examining cellular response to mechanical stimuli is an

important part of understanding the overall implications of vascular system restructuring, especially in the context of vascular diseases in which mechanical forces are altered.

## II. BACKGROUND

### Mechanotransduction in vascular smooth muscle cells

While mechanical forces are essential to the maintenance of normal vascular homeostasis, these forces also contribute to the pathologic alterations of the blood vessel in hypertension and other vascular disease. Vascular cells are exposed to various biomechanical stimuli including hydrostatic pressures, cyclic strains, and wall shear stresses caused by pulsatile blood flow [12]. It is important to understand how cells respond to mechanical forces in order to further our understanding of how disease modifies vascular structure. As mentioned previously, this is especially true of VSMC response to mechanical stimuli which is less well understood than the response of ECs.

Cellular response to mechanical stimuli via the process of mechanotransduction involves coordination between cell surface receptors, intracellular signaling pathways as well as rearrangement of the ECM and the cytoskeleton. Cell surface receptors that are involved in cell-ECM adhesion, trigger signaling processes within the cells and are a major component of the mechanotransduction process. Integrins and syndecans are two major gene families that make up ECM binding receptors found on the surface of vascular cells [9].

### Integrin and syndecan involvement in intracellular signaling pathways

Integrins have been shown to interact directly with ECM proteins in α and β heterodimeric pairs, forming focal adhesion complexes that transmit mechanical stimuli from the ECM to the interior of the cell [5,12,14,15]. In mammals, 19 α and 8 β subunit polypeptides have been discovered that combine to form 25 different heterodimers. Of

these 25 heterodimers only α1β1, α2β1, α3β1, α4β1, α5β1, α6β1, α7β1, α8β1, α9β1, αvβ1, αvβ3, αvβ5, and α6β4 have been found in VSMCs [17]. The extracellular domains of both integrin subunits bind to specific amino acid sequences on a variety of ECM ligands, such as fibronectin, vitronectin, collagen and laminin [18]. The short intracellular domain associates with a variety of cytoskeletal proteins and kinases, e.g., focal adhesion kinase (FAK) and c-SRC [19]. The role of integrins in mechanotransduction has been well characterized and has been shown to mediate cell movement, adhesion and death [20].

While previous work has focused on the role of integrin receptors in mechanotransduction, little is known about the role of the cell surface proteoglycans in these processes. Proteoglycans, especially those from the syndecan family, have been shown to collaborate with integrins in the formation of adhesion signaling complexes, organization of the cytoskeleton, and cell movement [9,21]. However, the exact nature of their interaction is highly complex and is not thoroughly defined. There are four syndecan genes expressed in vertebrates (syndecan-1, -2, -3, -4). Syndecan-4 is expressed throughout all cell types, while syndecan-1 is expressed in mesenchymal and epithelial cells, syndecan-2 is expressed in neuronal, mesenchymal and epithelial cells, and syndecan-3 is found in neuronal and musculoskeletal tissue [22]. All syndecans are transmembrane proteins that contain a short cytoplasmic tail, a single-span transmembrane domain, and a longer extracellular domain (Figure 1). This extracellular domain serves as a posttranslational attachment site for heparan sulfate (HS) chains that modulate cell response by interactions with growth factors and ECM proteins [10].

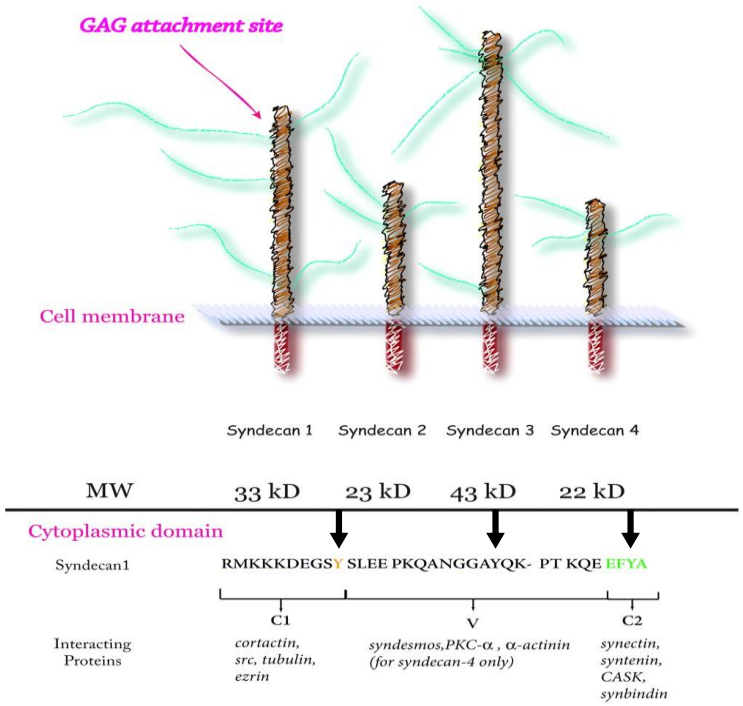


**Figure 1.** Structure of vertebrate syndecans illustrating extracellular, transmembrane and cytoplasmic domains [10]. Extracellular domains are shown with HS attachment sites. The intracellular domain is organized into the highly conserved C1 and C2 domains and the variable V domain.

Recently syndecans have been studied as to their specific impact on signaling. Syndecan-4 has been shown to modulate fibroblast growth factor 2 (FGF2) signaling which has implications in cell growth and angiogenesis (blood vessel growth). Syndecan-4 has also been shown to regulate cell migration, through interactions with β1 integrin, and to control adhesion through cystoskeletal modification [9]. The expression of syndecan-1 and syndecan-4 has also been shown to be upregulated during wound healing, especially in response to arterial injury [25] and myocardial infarction [26].

**Effects of mechanical stretch on VSMCs**

Mechanical strain mimicking that caused by pulsatile blood flow has been used to elucidate the effect of mechanical stimuli on VSMCs. Devices using indenters have been

used to apply both equibiaxial and uniaxial mechanical strain on to cells cultured on elastic membranes [12]. Cyclic stretch has also been applied to 3D cultures *in vivo* [23]. As a result integrins, receptor tyrosine kinases, and ion channels have all been implicated as stretch mechano-sensors. Mechanical stretch studies have determined that syndecan-4 and syndecan-1 are upregulated during mechanical stress [24]. While a body of work has been recently completed investigating the specific role of syndecan-4 in VSMC mechanotransduction [9] the specific role of syndecan-1 has yet to be studied extensively despite its implication in similar signaling pathways. An understanding of the involvement of syndecan-1 in VSMC mechanotransduction is therefore extremely relevant to the field. A more complete understanding of how mechanical forces affect vascular tissue may improve the ability to manage vascular disease.

This work focuses specifically on characterizing the role of the cell surface proteoglycan syndecan-1 in vascular cell mechanotransduction. To this end, mechanical forces were applied to rat aortae in an *ex vivo* culture system to examine the regulation of syndecan-1 expression. In addition, we isolated vascular smooth muscle cells from syndecan-1 knockout mice and transfected them with wild-type and mutated forms of syndecan-1 using lentiviral vectors. Wild-type (WT), syndecan-1 knockout (S1KO), transgenic human syndecan-1 (hSyn-1) and transgenic human syndecan-1 with a deleted cytoplasmic region (DELC), mouse vascular smooth muscle cells were cultured in plates with collagen coated silicone membranes. An *in vitro* mechanical loading system was used to subject the cells to 10% strain at 1 Hz biaxial or uniaxial loads for up to two hours. Immunostaining and western blot were used to evaluate the effects of syndecan-1

knockout on cytoskeletal rearrangement, adhesion, and phosphorylation of important signaling intermediates.

## III. MATERIALS AND METHODS

### Cell Transfection

Genes expressing human syndecan-1 and human syndecan-1 with deleted cytoplasmic region were cloned into a GFP expressing lentiviral vector (pLenti6.2-GW/EmGFP), which contained a constitutive promoter, using PCR. Vectors were transfected into a HEK293FT packaging cell line using Lipofectamine 2000 according to the manufacturers protocol (Invitrogen). The next day the media was changed to standard growth media. Viral particle containing media was collected after 48 hours of incubation with the cells. Cellular debris was removed by centrifuging the samples at 3000g for 5 minutes. Subconfluent VSMC cells isolated from syndecan-1 knockout mice were transduced with the viral particles in the presence of Polybrene (6 μγ/ml) for 24 hrs. The cells were then selected in Blasticidin (5 μg/ml) for 14 days to obtain stable cell lines.

### Cell Culture and Application of Mechanical Strain

Vascular smooth muscle cells from wild type (WT) mice as well as those from syndecan-1 knockout (S1KO) and S1KO transfected with human syndecan-1 (hSyn-1) as well as those transfected with human syndecan-1 with a deleted cytoplasmic region (DEL-C) were used between passage 5-7 and were maintained in MCDB 131 (Gibco/Invitrogen) with 10% fetal bovine serum and penicillin/streptomycin at 37°C in a humidified atmosphere at 5% $CO_2$. Cells were plated on collagen I coated silastic membranes (Speciality Manufacturing, Inc) or on ¼” strips of collagen coated silastic membranes in culture dishes. Biaxial mechanical strain was applied to membrane plated cells using a previously described membrane indenter device (Figure 2a), while uniaxial strain was applied to culture strips using an Electroforce® BioDynamic® Test instrument (BOSE),

shown in Figure 2b. All mechanical strain was applied with a maximal strain of 10% at a frequency of 1 Hz. Strain was performed for 4 hours and 12 hours for biaxial and uniaxial loading of cells cultured on silicone strips, respectively.

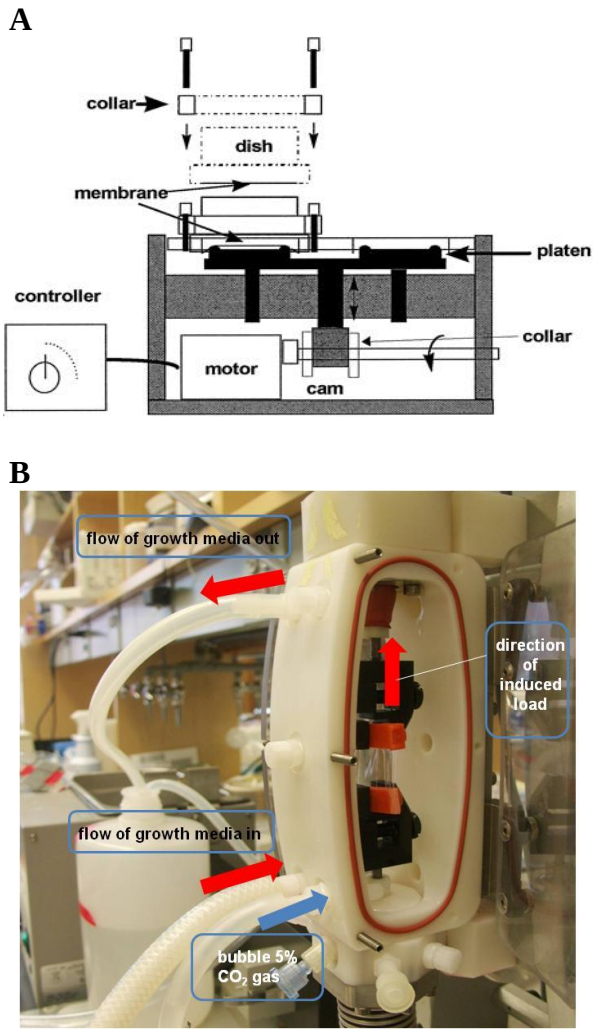


**Figure 2.** (A) Diagram of biaxial loading device similar to loading device used in this experiment [27], (B) View of BioDynamic test device used for uniaxial loading. Cell culture medium is flowed through test device with a 5% $CO_2$ bubbler to pH balance growth media.

**Immunocytochemical Staining**

Cells were washed twice in PBS fixed in 4% paraformaldehyde for 10 minutes then blocked for 1 hour in 20% FBS. Primary antibodies for F-actin, Paxillin, Phospho-SRC and Ezrin (Santa Cruz Biotech) were added at a 1:250 ratio in 1% BSA in PBS overnight at 4°C. Secondary antibodies labeled with Alexa Fluor 594 or 488 (Invitrogen) were added and samples were incubated for 2 hours at room temperature. Cytoskeletal actin staining was achieved using Alexa Fluor 594 conjugated rhodamine-phalloidin at 1:250 ratio in 1% BSA in PBS for 1 hour. After washing with PBS samples were mounted with DAPI containing anti-fade media (Vector Labs) and imaged.

**Ex-Vivo Model of Syndecan-1 Expression After Mechanical Strain**

Full aortae were obtained from wild type laboratory rats using procedures and protocols approved by the Animal Care and Use Committee of the Massachusetts Institute of Technology and consistent with NIH and American Physiological Society standards. Aortae were strained at 20% for 2 hours under culture conditions using Electroforce® BioDynamic® Test instrument (BOSE) and then flash frozen in isopentane that was previously cooled in liquid nitrogen. Samples were then stored at -80°C.

**Immunohistochemistry**

Frozen aortae were cryosectioned, placed onto slides, hydrated with PBS, then blocked for 40 minutes in 20% FBS in PBS. Primary syndecan-1 antibody (CD138) was added at 1:25 and contacted overnight at 4°C. Secondary Alexa Fluor 594 (Invitrogen) was added at 1:400 and incubated for two hours at room temperature. After washing with PBS, samples were mounted with DAPI containing anti-fade media (Vector Labs) and imaged. Fluorescent intensity of cross sections was measured using software provided with the fluorescent scope.

**Cell and Tissue Lysis and Western Blotting**

Cells from VSMC lines and sectioned aortae from rats were lysed in a solution containing 20mM Tris, 150mM NaCl, 1% Triton, 0.1% SDS, 2mM sodium orthovandanate, 2 mM PMSF, 50mM NaF and a complete protease inhibitor tablet (Roche). Digested samples were examined using a BCA Assay (Pierce) to determine relative protein concentration then normalized and run on NuPAGE® Novex® Bis-Tris Gels (Invitrogen) with MOPS SDS buffer and transferred to membranes using the included dry transfer system. Membranes were blocked for one hour in 5% nonfat milk in PBS with 0.01% tween-20 (PBST) and exposed to phospho-ERK, phospho-AKT, syndecan-1 and β-actin (for loading control) antibodies overnight at 4°C. Membranes were then washed with PBST and incubated for two hours at room temperature with a secondary antibody and detected using chemiluminescence.

## IV. RESULTS

### Syndecan-1 knockout increases actin stress fibers and formation of focal adhesion sites in response to mechanical strain.

We examined the role of syndecan-1 in cytoskeletal remodeling and formation of focal adhesion sites in response to mechanical strain. VSMCs were biaxially loaded at 10% strain for four hours using the previously described membrane indenter device (Figure 2A). Following the treatment, staining with F-actin and ezrin was performed to detect actin fiber distribution (Figure 3). Qualitative analysis determined an increase of cortical stress fiber formation in syndecan-1 modified cell lines, including overexpressing hSyn-1, in comparison to loaded WT cells. F-actin staining also indicates a pronounced increase in intracellular stress fiber formation in proximity to the nuclear domain in S1KO and DEL-C cells, which is not evident in WT and hSyn-1 cell lines in response to treatment.

Further qualitative evidence of increased cytoskeletal formation in response to loading is illustrated by ezrin expression. Ezrin has been found to have binding domains for both the actin skeleton and membrane components implicated in mechanotransduction [27]. Staining indicates increased expression of ezrin in the intracellular space proximal to the nuclear domain in SIKO and DELC cell lines post treatment (Figure 3). This expression is consistent with the elevated expression of actin in similar regions described previously.

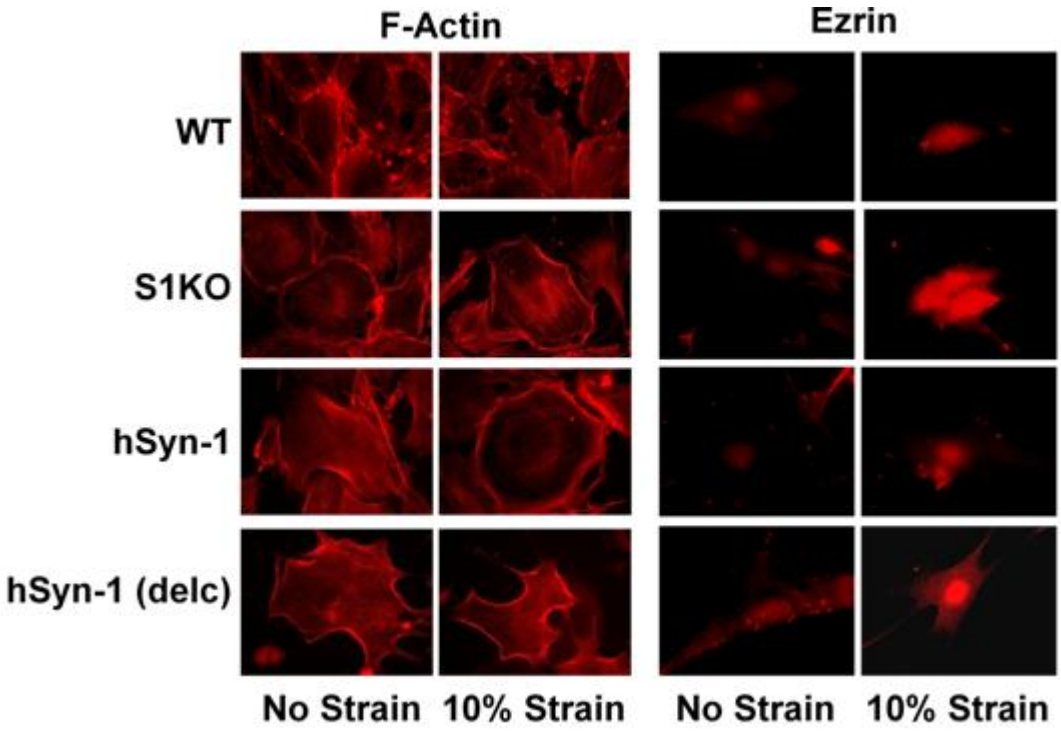


**Figure 3.**Syndecan-1 knock out or deletion of the cytoplasmic region causes an increase in actin stress fibers and intracellular ezrin expression in proximity to the nuclear domain.

Staining of paxillin in the four cell lines indicated an obvious increase in the formation of focal adhesion sites (punctuate dots) in S1KO or DELC cell lines even before strain (Figure 4). WT cells increase focal adhesion sites at the leading edge of migration after treatment while overexpressing hSyn-1 appears unable to form prominent focal adhesion sites even after treatment. Phosphorylation of SRC, a tyrosine receptor kinase, is essential in formation of focal adhesion complexes [28]. Staining of phospho-SRC follows a similar trend to that of paxillin in the four experimental cell types in response to strain (Figure 4). SIKO and DELC cell lines exhibit pronounced phospho-SRC expression in response to loading which is indicative of focal adhesion site formation. Again, S1KO exhibits substantial focal adhesion formation even before treatment.

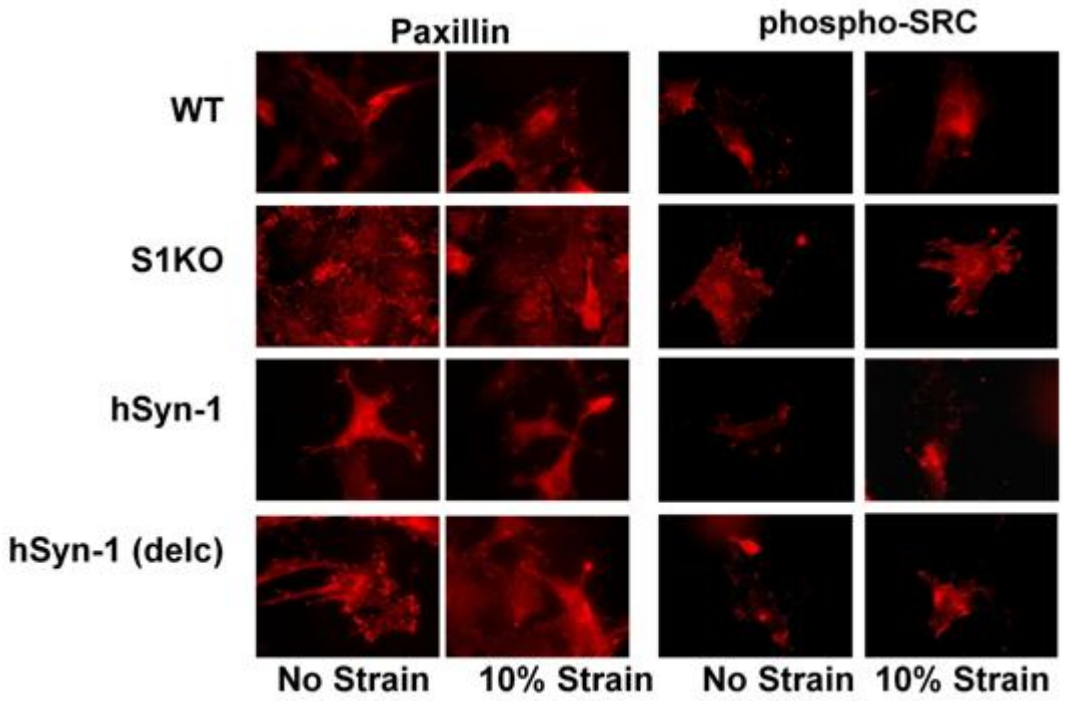


**Figure 4.** Staining indicates increased focal adhesion site formation in S1KO and DELC cell lines as evidenced by paxillin and phopho-SRC expression after treatment with 10% mechanical strain for a duration of four hours.

**Compromised syndecan-1 increases phospho-ERK and phospho-AKT in response to mechanical strain.**

Vascular smooth muscle cells were treated with 10% strain for four hours and were then scraped off of plates and lysed before performing western blots. Western blots were run with antibodies detecting phospho-ERK and phospho-AKT. Increased phospho-ERK expression is implicated in increased DNA synthesis and is therefore a marker of cell proliferation [29]. Increased phospo-AKT expression is linked to pathways that result in increased cell survival [30]. Results indicate increased expression of phosph-ERK amongst all cell lines in response to loading. The increased expression was especially substantial in cells without syndecan-1 (SIKO) or where the cytoplasmic region of

syndecan-1 was deleted (DELC) (Figure 5). Phospho-AKT expression was analyzed in all four cell lines before and after loading and expression appears to be higher in DELC than all other cell lines after loading, though this result was not determined to be statistically significant as quantitative analysis of band intensity was not performed.

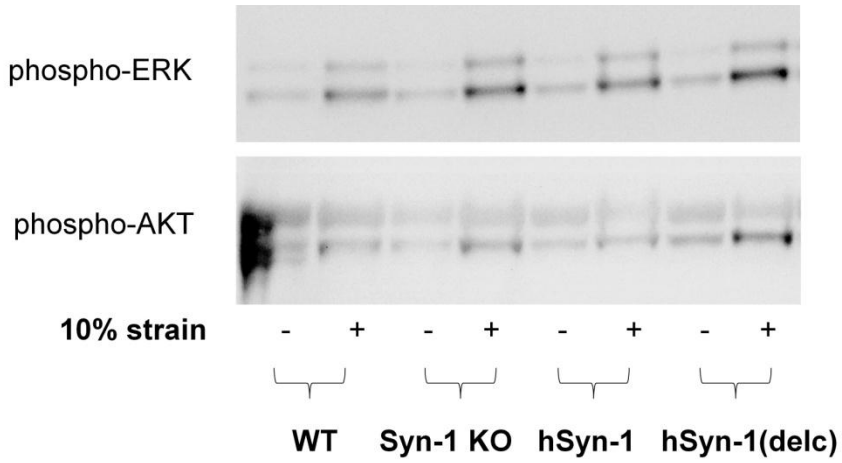


**Figure 5.** Syndecan-1 knock out or deletion of cytoplasmic domain facilitates increased expression of intermediates in proliferation and cell survival pathways. These changes are evidenced by increased expression of phospho-ERK and phospho-AKT, respectively.

**Knock out of syndecan-1 alters cytoskeletal alignment in response to uniaxial mechanical strain.**

All four established cell lines were cultured on silicone rubber strips and subjected to 10% maximum strain for 12 hours under culture conditions using an Electroforce® BioDynamic® Test Instrument (BOSE). Cells were then fixed and stained for F-actin and paxillin (Figure 6). Staining with F-actin indicates a dramatic difference in actin stress fiber formation between WT and S1KO cells in response to strain. Actin stress fibers in WT cells appear to be in the process of aligning perpendicular to the direction of strain and are in less abundance compared with S1KO or DELC cells. SIKO and DELC cells not only have greater stress fiber formation that WT cells they also have pronounced areas of crossed actin fibers, which were not observed in WT cells. Staining with paxillin illustrates greater expression in S1KO and DELC cell lines in response to strain compared with expression in WT and hSyn-1 cell lines.

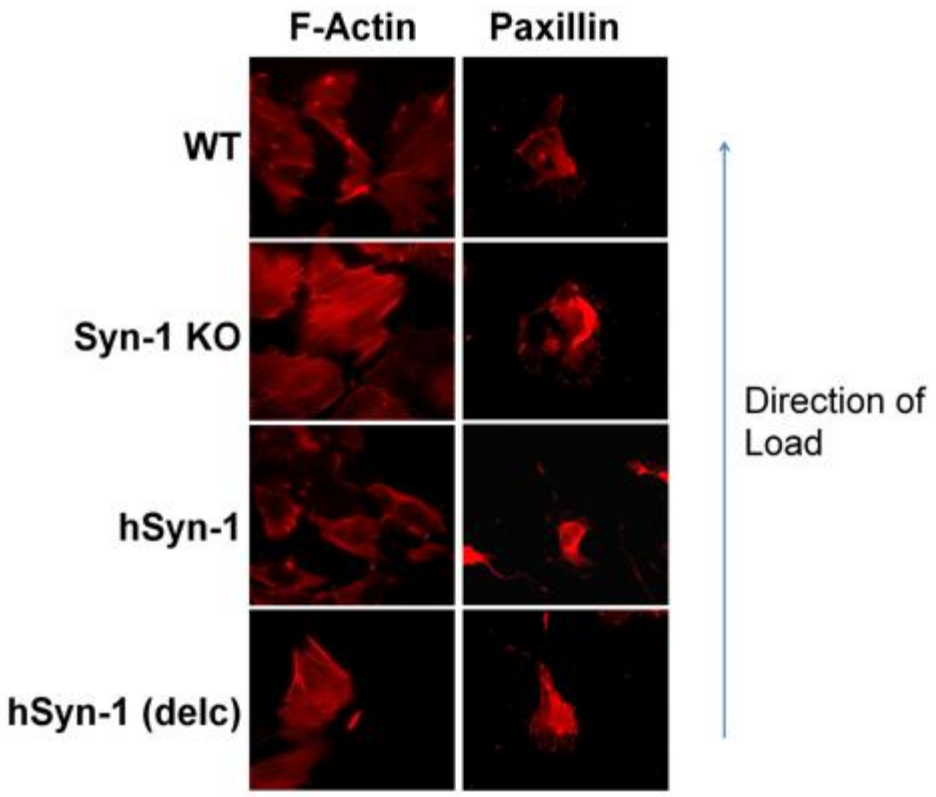


**Figure 6.** Cell lines with absent or compromised syndecan-1 exhibit differences in cytoskeletal rearrangement after treatment with uniaxial mechanical strain, in comparison with WT cells. Notably, areas of crossed actin fibers are observed in S1KO and DELC cell lines.

**Uniaxial loading of vascular tissue yields inconclusive effects on syndecan-1 expression.**

Aortae from laboratory rats were harvested and subjected to 20% strain for two hours using an Electroforce® BioDynamic® test instrument. Cryosections were stained for syndecan-1 expression and were also examined for syndecan-1 expression using western blot. Hematoxylin and eosin stained cross sections illustrate significant tissue change after treatment with 20% strain (Figure 7a). Representative sections of loaded and unloaded syndecan-1 stained samples appear similar as both display pronounced regions of syndecan-1 expression (Figure 7b).

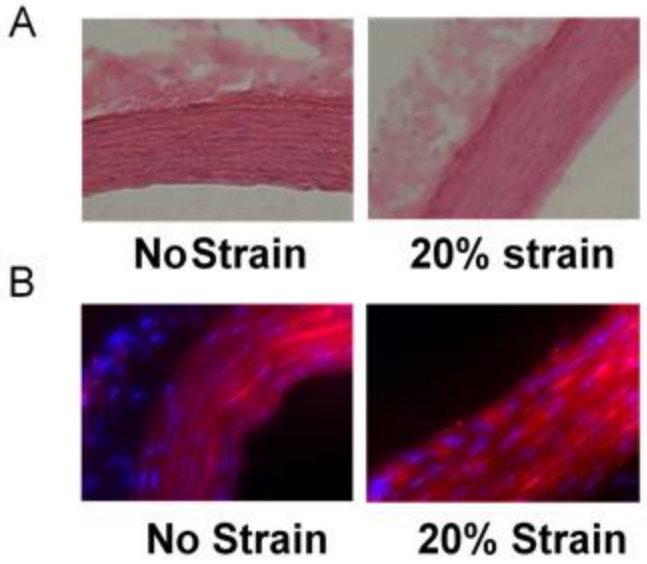


**Figure 7.** Representative (A) hematoxylin and eosin stained and (B) syndecan-1 immunostained aorta cross sections with and without treatment at 20% uniaxial mechanical strain.

A quantitative analysis of fluorescent intensity from loaded and unloaded aorta cross sections indicated a significant increase of syndecan-1 expression after loading in only one of four animals (Figure 8a). This observation was supported by western blot where samples taken from that same animal displayed increased expression after loading while the samples from the other animals did not (Figure 8b).

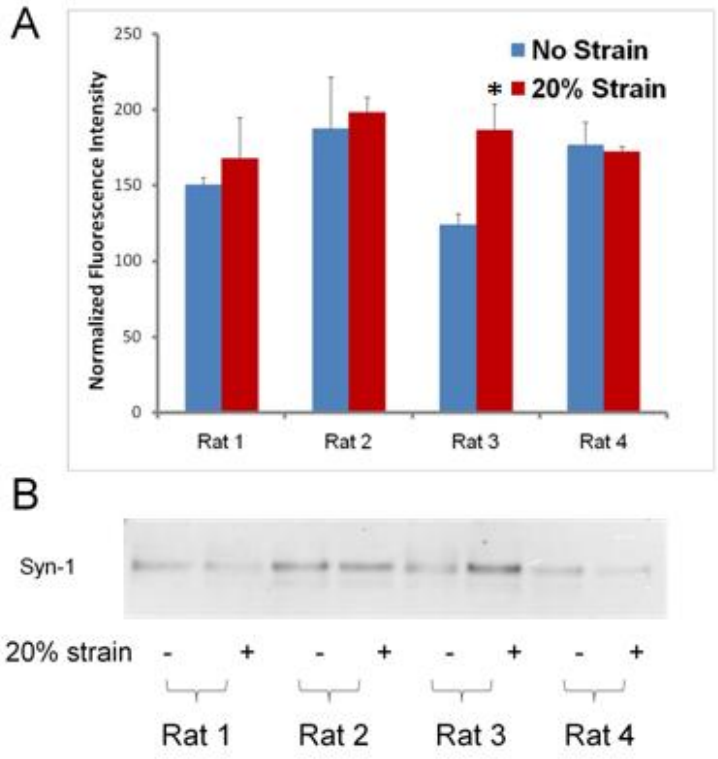


**Figure 8.** (A) Normalized fluorescence intensity analysis of syndecan-1 immunostained aortae indicates significant increase in syndecan-1 expression after loading in only one test animal. Error bars plotted as + 2 standard deviation. Significant difference is based on 95% CI, denoted by (*). (B) Western blot analysis to detect syndecan-1 expression supports observation of increase in syndecan-1 expression after loading in samples harvested from only one animal.

## V. DISCUSSION

Mechanotransduction pathways that convert external mechanical stimuli to intracellular signaling cascades are essential for maintaining vascular homeostasis [4]. Understanding the signaling pathways and the molecular basis for these interactions is an important part of combating vascular diseases; especially those, such as hypertension, in which mechanical forces are altered. Previous studies have implicated integrins in mechanotransduction pathways that govern cell adhesion, movement, proliferation and death in VSMCs. Syndecans have been studied with respect to their role in signaling pathways, but not explicitly those involved in mechanotransduction [9]. This work begins to characterize the role that a specific syndecan, syndecan-1, plays in mechanotransduction pathways that govern adhesion, mobility, proliferation and survival.

Our biaxial loading studies of VSMCs indicate that cells with S1KO and DELC have pronounced increase in F-actin and ezrin expression in the intracellular space, in comparison to WT and hSyn-1 expressing cells, in response to 10% mechanical strain for four hours. These results demonstrate that elimination of syndecan-1 or deletion of the cytoplasmic region, appears to increase the synthesis of actin cytoskeleton in response to strain. This is consistent with previous studies that have demonstrated elevated expression of syndecan-1 is associated with down regulation of cell motility and growth [9], of which cytoskeletal synthesis and degradation plays an important part. This study also demonstrated that formation of focal adhesions is increased dramatically in cells with compromised syndecan-1 or syndecan-1 knock out after mechanical strain, in comparison to those cells expressing syndecan-1. Together these results implicate

syndecan-1 as an important regulator of adhesion site formation and synthesis of actin cytoskeleton in response to strain.

In this study we also demonstrate that S1KO and DELC also have increased phospho-ERK and phospho-AKT expression in response to 10% biaxial mechanical strain. These data indicate that decreased syndecan-1 expression is linked with increased DNA synthesis and upregulation of cell survival pathways in response to mechanical strain. We interpret these results to describe the normal function of syndecan-1 as an inhibitor of cellular signaling pathways that govern proliferation and survival in response to mechanical strain. Our observations are consistent with previous studies which show absence of syndecan-1 is consistent with increase in cellular proliferation, especially in metastatic tumor cells [31].

Uniaxial loading studies on cultured cells were performed to investigate cytoskeletal rearrangement in response to strain. We observed substantial differences in actin stress fiber alignment between different cell types in response to mechanical strain. Notably, S1KO and DELC cell lines appeared to have a greater amount of actin fibers than in WT or hSyn-1 cells after loading. Further, we noted that S1KO and DELC cell lines had pronounced areas of crossed actin fibers after strain, in comparison to WT cells that displayed stress fibers organized mostly perpendicular to the direction of strain. These results are consistent with our previous conclusion that syndecan-1 acts as an inhibitor of vascular remodeling in response to strain. However, the observation of crossed actin fibers during alignment illustrates that presence of syndecan-1 might be essential for proper cytoskeletal alignment in response to changes in mechanical forces.

Our study of mechanical strain of vascular restructuring ex-vivo produced inconclusive results. Intensity analysis of syndecan-1 stained aortae indicated syndecan-1 expression is not increased in vascular tissue after loading in all samples using the prescribed methods. This analysis did indicate, however, that one of the four aortas displayed increased expression after loading to a statistically significant degree. These same results were confirmed by western blot. Further experimentation should be performed using this model to determine syndecan-1 expression in vascular tissue in response to strain before any conclusions can be made.

**Summary and Future Work**

In summary, we have established that syndecan-1 appears to act as an inhibitor of cellular adhesion, movement, proliferation and survival in strain induced mechanotransduction pathways in VSMCs. Our study also indicates that syndecan-1 may play an important role in the coordination of stress fiber realignment in response to mechanical strain. Future work will focus on adding quantitative techniques to confirm qualitative observations formed from immunostaining. In particular, fluorescent intensity analysis will be performed on immunostained replicates to evaluate observations of increased expression of selected markers within cells. Further, BRDU incorporation will be implemented as a quantitative method to determine levels of cellular proliferation in response to strain.

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