Syndecan-1
An Inhibitor of Arterial Smooth Muscle Cell Growth and Intimal Hyperplasia

Nozomi Fukai, Richard D. Kenagy, Lihua Chen, Lu Gao, Guenter Daum, Alexander W. Clowes

Objective—Arterial injury induces smooth muscle cell (SMC) proliferation, migration, and intimal accumulation of cells and extracellular matrix. These processes are regulated by the administration of the glycosaminoglycans heparin and heparan sulfate, but little is known about the role of endogenous heparan sulfate proteoglycans in the vessel wall. We investigated the response to carotid injury of syndecan-1–null mice to assess the function of one of a conserved family of transmembrane heparan and chondroitin sulfate proteoglycans.

Methods and Results—Syndecan-1–null mice developed a large neointimal lesion after injury, whereas wild-type mice made little or none. This was accompanied by a significant increase in both medial and intimal SMC replication. Cultured syndecan-1–null SMCs showed a significant increase in proliferation in response to PDGF-BB, thrombin, FGF2, EGF, and serum. In response to thrombin, PDGF-BB, and serum syndecan-1–null SMCs expressed more PDGF-B chain message than did wild-type SMCs. Downregulation of PDGF-BB or PDGFRβ inhibited thrombin-, PDGF-BB–, and serum-induced DNA synthesis in syndecan-1–null SMCs.

Conclusions—These results suggest the possibility that syndecan-1 may limit intimal thickening in injured arteries by suppressing SMC activation through inhibition of SMC PDGF-B chain expression and PDGFRβ activation. (Arterioscler Thromb Vasc Biol. 2009;29:00-00.)

Key Words: syndecan • platelet-derived growth factor • smooth muscle • arterial injury

Arterial injury induces smooth muscle cells (SMCs) to proliferate, migrate, and accumulate in the intima. These processes may be modulated by components of the extracellular matrix (ECM), particularly heparin and the related glycosaminoglycan heparan sulfate. These glycosaminoglycans inhibit arterial SMC migration and proliferation and alter SMC ECM composition.1–3 Although perlecan, an ECM-associated heparan sulfate proteoglycan (HSPG), has been shown to inhibit SMC proliferation and intimal thickening,4,5 the role of other HSPGs in the injured vessel wall has not been defined.

Syndecan-1 is a member of a conserved family of 4 heparan- and chondroitin-sulfate–containing transmembrane HSPGs (syndecans 1 to 4) that are expressed in a developmental-, cell-type–, and tissue-specific manner.6 Syndecans bind various components of the ECM and are important regulators of cell–cell and cell–ECM interactions. All syndecans are normally expressed in the artery. After injury, the levels of mRNA for syndecan-1 slowly rise and peak at about 2 weeks, whereas syndecan-4 peaks at 4 hours, falls toward baseline, and then rises again along with syndecan-1.7,8 Although all syndecans can bind heparin-binding growth factors and syndecan-4 is known to be required for thrombin-induced migration and proliferation in human aortic SMCs,9 little is known about the role of syndecan-1 in SMC function. In addition, although it is known that syndecan-4 null mice exhibit a delayed skin-wound healing response and syndecan-1–null mice develop a hypocellular scar,10,11 no studies have been performed to define role for syndecans in the response to vascular injury. Therefore, we have investigated the effects of carotid artery injury in wild-type and syndecan-1–null mice and the growth and migratory properties of vascular SMCs from these mice.

Materials and Methods

Cell culture supplies were purchased from Invitrogen Corporation. Fetal bovine serum (FBS) was purchased from Atlantic Biological. Polymerase chain reaction (PCR) primers and small interfering RNAs were purchased from Invitrogen Corporation. The following reagents were obtained from the company indicated: Platelet-derived growth factor (PDGF)-BB and mouse fibroblast growth factor 2 (FGF2) from R&D systems, human α-thrombin from American Diagnostica, a phospho-tyrosine antibody (clone 4G10) from Upstate Biotechnology, an antibody against PDGF receptor β (PDGFRβ) from Santa Cruz Biotechnology, an antibody against human α-smooth muscle actin from DAKO, and an antibody against syndecan-1 from BD Pharmingen. [3H]-Thymidine was purchased from NEN Life Science Products. Other chemicals were purchased from Sigma.
Animals and Surgical Procedure
Breeding pairs of syndecan-1–null mice (C57BL/6 background) originally developed by Merton Bernfeld’s laboratory were generously provided by William C. Parks (University of Washington, Seattle). The genotype of offspring was determined by PCR analysis, and 8- to 9-week-old littermate null or wild-type males were used for all experiments. The left common carotid artery was ligated with a 0-0 suture near the carotid bifurcation. After 7, 14, and 28 days the carotid arteries were flushed via the left ventricle with PBS followed by 4% paraformaldehyde in PBS. The carotid arteries were removed and placed in fixative either overnight (for morphology) or for 3 hours (for BrdU staining). To determine SMC replication, mice were injected intraperitoneally with bromo-deoxyuridine (BrdU; 30 μg/g body weight) at 1, 9, and 17 hours before sacrifice at 7 and 14 days after injury. All studies were performed within the guidelines for animal experimentation at the University of Washington and approved by the University of Washington institutional review board.

Morphological Measurements and Immunohistochemistry
Arteries were cut at the midpoint, and both pieces were embedded together in paraffin. Histological cross sections were cut from the midpoint of every 50 sections (a total of 40 sections) and then stained with hematoxylin and eosin. Cross-sectional area of intima and media of 20 sections/mouse was determined using SPOT software (Diagnostic instruments Inc). Sections were stained for BrdU using the streptavidin-biotin/horseradish peroxidase method (Vecstain Elite ABC, Vector Laboratories). Hematoxylin-positive (total) and BrdU-positive (replicating) cells were counted in 6 sections per animal. Intimal and medial nuclear number per cross-section (and nuclear number/mm²) were also determined in 20 sections per mouse.

Mouse Arterial Smooth Muscle Cell Isolation
SMCs were isolated from adult male mouse aorta using an enzyme mix (2 mg/mL BSA, 4 mg/mL collagenase, 0.375 mg/mL soybean trypsin inhibitor, and 0.125 mg/mL elastase type III in Hanks’ balanced salt solution). After 10 minutes of incubation, the adventitial layer was removed and the remaining tissue was incubated at 37°C for further 2 hours; cells were then collected. Cells were maintained in 10% FBS in Dulbecco modified Eagle’s medium (DMEM) and used up to the thirteenth passage. Cells were characterized as smooth muscle cells by SM α-actin expression.

SMC Growth and DNA Synthesis
Cells at 60% to 80% confluence were changed to serum-free medium. After 48 hours the medium was changed again for another 24 hours. Growth factors were added without a change of medium. [3H]-thymidine (0.5μCi/mL) was added 4 hours after addition of growth factors. After a 24-hour exposure to [3H]-thymidine, the cells were harvested for determination of DNA synthesis as previously described. Two methods were used to quantify replication. First, cells were plated at 5000 cells per well in 6-well plates in growth medium (10% FBS), which was changed after 2 days. Cells were harvested daily and counted from triplicate plates using a hemocytometer. In the second method used in siRNA experiments, cells were seeded at 6000 cells per well in 24-well plates in growth medium (10% FBS), which was changed after 2 days. Cells were harvested at day 3, 7, and 14 and counted from triplicate plates using a hemocytometer. For attachment assays, adherent cells were washed twice with phosphate-buffered saline, and the MTT was solubilized with 100 μL of DMSO by shaking for 5 minutes and quantitated by measuring absorption at 560 nm.

SMC Migration and Attachment
Microchemotaxis assays were performed with 48-well chambers (Neuro Probe) and polycarbonate filters (10 μm pores) coated with 100 μg/mL monoclonal bovine type I collagen (BD Biosciences). Because syndecan-1 is shed by trypsin, we used 2 mmol/L EDTA in Ca²⁺/Mg²⁺ free PBS to release cells from the culture dishes. Suspended cells were added to the upper chamber (25,000 cells per well), and chemotactants or DMEM were added to the lower chamber. After 5 hours the migrating cells were fixed and stained with Diff-Quick staining solution (Baxter), and the number of cells per high-power field (×100) that had migrated across the membrane was determined.

For attachment assays, adherent cells were washed with versene (1 mmol/L EDTA in PBS), suspended with trypsin, washed twice with soybean trypsin inhibitor (STI, 0.5 mg/mL in PBS), and resuspended in DMEM containing 1 mg/mL BSA. Cells (3×10⁴ cells per well) were plated in 96-well plates coated with 100 μg/mL of polymeric type I collagen or 10 μg/mL fibronectin. After 20 minutes at 37°C, the wells were rinsed with warm PBS to remove nonattached cells, and the remaining cells were stained for 15 minutes with 0.2% crystal violet in 20% methanol. After 5 washes with water, the dye was solubilized by adding 0.1 mol/L Na citrate 50% ethanol (pH 4.2) and quantified by measuring absorbance at 550 nm using an ELISA plate reader. Assays were performed in triplicate.

Quantitative PCR
Total RNA from 5 to 8 pooled carotids or from SMCs was isolated using the RNeasy Mini Kit (Qiagen) and was reverse transcribed using Superscript reverse transcriptase (Promega). Quantitative polymerase chain reaction (qPCR) of syndecans-1, -2, -3, -4, PDGF-B, and ARBP was performed using SYBR Green (Molecular Probes) with the Fast Real-Time PCR 7500 system (Applied Biosystems). The forward and reverse primers used (Invitrogen) are listed in supplemental Table 1 (available online at http://atvb.ahajournals.org). SMCs isolated from the syndecan-1–null mouse did not express syndecan-1 mRNA or protein, but did express syndecans-2, -3, and -4 (supplemental Figure 1).

Transfection of siRNA Targeting Syndecan-1 and PDGFRβ
For DNA synthesis experiments, cells were suspended in 10% FBS and seeded at 6250 cells per cm² in 12-well dishes. Then cells were transfected with siRNAs (5 nmol/L) using HiPerFect Transfection Reagent (Qiagen) according to the manufacturer’s instructions. The next day medium was changed to serum-free DMEM, left overnight, and changed again. After 2 days growth factors were added followed by [3H]-thymidine. In addition, some samples were extracted, subjected to 7.5% SDS-PAGE, and transferred to nitrocellulose membranes. Immunodetection of PDGFRβ was performed using an antipeptide antibody (Santa Cruz, sc-432) and enhanced chemiluminescence (Amersham). Separate samples were used to extract RNA for qPCR for syndecan-1. For cell proliferation assays, transfections were carried out as described above with the different growth factors (eg, 1% FBS ± thrombin or PDGF-BB) and left for 2 days. Medium was then replaced with fresh medium with or without growth factors.

Statistical Analysis
Data are presented as mean±SEM. Means were compared between groups using multiple analysis of variance or t test depending on the number of groups to be compared. In the case of nonnormal distributions, nonparametric methods were used. P<0.05 was considered significant.

Results
Intimal Thickening in Syndecan-1–Null Mice After Carotid Ligation
Fourteen days after ligation, neointimal lesions were present in syndecan-1–null arteries but not in wild-type arteries, and these lesions continued growing up to 28 days (Figure 1A). The intima/media ratio of injured syndecan-1–null carotids was significantly greater than that of wild-type arteries (Figure 1B), as was the number of intimal cells (Figure 1C). The luminal area was decreased in the syndecan-1–null
arteries at 28 days ($P<0.055$; supplemental Figure II). However, total vessel area (measured as the area inside the external elastic lamina) and medial area were not different, and therefore the loss of luminal area was accounted for by the increase in intimal area (supplemental Figure II). Medial cell number remained constant throughout the experiment in wild-type carotids, but was significantly higher in syndecan-1–null arteries 28 days after ligation (Figure 1D). These medial cells stained positive for smooth muscle actin and negative for Mac-2 (data not shown). In conjunction with the growth of the neointima, significantly increased rates of SMC BrdU incorporation were observed at 7 and 14 days in the intima and media of syndecan-1–null carotids compared to wild-type carotids (Figure 2A and 2B).

**Proliferation and Migration of Syndecan-1–Null SMCs**

Because we observed increased rates of proliferation in vivo, we investigated the response of wild-type and syndecan-1–null SMCs to multiple growth factors and chemoattractants involved in the vascular response to injury, such as PDGF, FGF2, EGF, and thrombin. PDGF-BB, EGF, and FGF2 increased DNA synthesis ($[^3]H$)-thymidine incorporation in wild-type and syndecan-1–null SMCs, whereas syndecan-1–null SMCs exhibited higher rates of DNA synthesis compared to wild-type SMCs in response to all of these growth factors (about 2-fold higher for wild-type for FGF2, 3-fold for EGF, and about 5-fold for thrombin and PDGF-BB; Figure 3A). Cell proliferation over 4 days in response to serum (Figure 3B), PDGF-BB, thrombin, and FGF2, but not EGF (supplemental Figure IIIA and IIIB) was greater for syndecan-1–null SMCs than for wild-type SMCs. Importantly, knockdown of syndecan-1 in wild-type SMCs by siRNA also increased cell proliferation in response to serum (Figure 4A). Conversely, transfection of syndecan-1 into syndecan-1–null SMCs inhibited cell proliferation (Figure 4B); these results confirm the difference observed between syndecan-1–null and wild-type SMCs. The siRNA to syndecan-1 decreased syndecan-1 mRNA by 90% (supplemental Figure IV).

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**Figure 1. Morphometric analysis of wild-type and syndecan-1–null carotid arteries after ligation injury.** A, Photomicrograph of histological cross-sections of carotid arteries from wild-type and syndecan-1–null mice 4 weeks after total ligation. Sections were stained with hematoxylin and eosin. Arrow points to the internal elastic lamina. 200 x (B) Intima/media ratio. B, Intimal cell number per cross-section. C, Medial cell number per cross-section. *P<0.05 wild-type vs null; for wild-type and null, respectively: n = 6 and 8 on day 7; 6 and 8 on day 14; and 6 and 14 on day 28.

**Figure 2. DNA synthesis in wild-type and syndecan-1–null carotid arteries after ligation injury.** The BrdU index of cells in the intima (A) and media (B) was determined 7 and 14 days after carotid ligation. *P<0.05 wild-type vs null. n = 5 to 8.

**Figure 3. Proliferative response of wild-type and syndecan-1–null SMCs in response to different growth factors.** A, DNA synthesis in response to stimulation of wild-type and syndecan-1–null SMCs with either thrombin (10 nmol/L), PDGF-BB (10 ng/mL), or FGF2 (10 ng/mL). *P<0.05 growth factor vs control; †P<0.05 growth factor in syndecan-1–null vs wild-type; n = 4 to 7 experiments. B, Wild-type and syndecan-1–null SMC proliferation in response to stimulation with 10% serum. *P<0.05 wild-type vs syndecan-1–null. n = 3.
In response to PDGF-BB, syndecan-1–null SMCs migrated in a microchemotaxis chamber about 50% faster than wild-type SMCs (supplemental Figure V). Neither thrombin nor FGF2 stimulated migration of wild-type or syndecan-1–null SMCs. Because changes in cell adhesion could affect migration, we investigated attachment of the syndecan-1–null SMCs to collagen or to fibronectin. There was no significant difference in attachment of syndecan-1–null and wild-type SMCs to either collagen or fibronectin (supplemental Table II).

**PDGF-B Chain Expression Is Necessary for the Proliferation of Syndecan-1–Null SMCs in Response to Serum, Thrombin, and PDGF-BB**

We have previously shown that SMC proliferation in response to thrombin is mediated by the heparin-sensitive costimulatory factors FGF2 or HB-EGF. However, we found that thrombin-mediated stimulation of wild-type or syndecan-1–null SMCs to collagen or to fibronectin was insensitive to heparin (data not presented). Therefore, we tested the hypothesis that increased proliferation observed in the syndecan-1–null cells in response to thrombin results from increased PDGF-B chain induction, because PDGF-BB–mediated proliferation can be insensitive to heparin. We observed that PDGF-B is induced by thrombin in wild-type and syndecan-1–null SMCs but to a much greater extent in syndecan-1–null SMCs (Figure 5). PDGF-BB also induced PDGF-B expression in wild-type and syndecan-1–null SMCs, but to a lesser extent and with different kinetics (Figure 5). In contrast, neither FGF2 nor EGF altered PDGF-B expression over 6 hours (Figure 5).

To determine the role of PDGF-B and PDGFRβ in growth factor–induced SMC proliferation, we downregulated PDGF-B or PDGFRβ using siRNA. This treatment decreased levels of PDGF-B mRNA by 90% in wild-type and syndecan-1–null SMCs 6 hours after addition of growth factors (DNA synthesis experiments; supplemental Figure VIA) and 4 days after exposure to growth factors (cell proliferation experiments; supplemental Figure VIB). Treatment of syndecan-1–null cells with PDGF-B siRNA inhibited thrombin-, PDGF-BB–, and serum-mediated, but not FGF2– or EGF-mediated, DNA synthesis. Treatment of wild-type SMCs with siRNA to PDGF-B did not alter DNA synthesis in response to any of the growth factors tested (Figure 6A). Proliferation of syndecan-1–null SMCs as measured by MTT assays in response to thrombin, PDGF-BB was also inhibited by siRNA to PDGF-B (supplemental Figure VII). Treatment of cells with PDGFRβ siRNA decreased PDGFRβ protein by >90% (supplemental Figure VIC) and significantly inhibited thrombin- and PDGF-mediated DNA synthesis in the syndecan-1–null SMCs but had no effect on EGF- or FGF2-mediated DNA synthesis (Figure 6B).

**Discussion**

This study shows that the deletion of syndecan-1 converts an artery unresponsive to injury to an artery that develops large neointimal lesions and thus identifies syndecan-1 as an important inhibitory factor for lesion formation. Proliferation of syndecan-1–null SMCs is increased in vivo after injury in comparison to wild-type SMCs. It is also increased in vitro in response to PDGF-BB, FGF2, EGF, serum, and thrombin. Thus, syndecan-1 inhibits the SMC response to major factors previously documented to be involved in intimal hyperplasia.

**Costimulatory Growth Factor Pathway Inhibited by Syndecan-1 in SMCs**

We have observed that thrombin, PDGF-BB, and serum increase PDGF-B induction, an effect that is enhanced in the
absence of syndecan-1. Our data indicate that PDGF-B and PDGFRβ mediate the enhanced effect of these growth factors on DNA synthesis in the syndecan-1–null SMCs. In contrast, the enhanced EGF- and FGF2-mediated DNA synthesis in syndecan-1–null SMCs is not mediated by PDGF-B. It should be noted that these observations are unusual. PDGF-A is regulated in SMCs by a number of factors including thrombin, and PDGF-C is regulated by FGF2. PDGF-B has been detected in cultured neointimal and newborn SMCs, but not in adult SMCs. In addition, PDGF-B mRNA has been variably detected in vivo in injured rat carotid arteries. Several laboratories including our own have shown that thrombin-induced mitogenesis requires the activation of a secondary growth factor-receptor system. Both heparin-binding EGF-like growth factor (HB-EGF) and FGF2 have been shown to mediate the activity of thrombin, and both are blocked by heparin. We have now added PDGF-B chain to this list of secondary growth factors. We have observed considerable variability in the sensitivity of thrombin-mediated DNA synthesis to heparin and an EGF kinase inhibitor among SMCs isolated from individual nonhuman primates and humans (unpublished data and ), suggesting that the PDGF-B pathway may be important.

Possible Mechanisms for the Inhibitory Effect of Syndecan-1

Our data indicate that there are at least 2 inhibitory syndecan-1 pathways. The first, which inhibits the mitogenic actions of thrombin, PDGF-B, and serum, acts via inhibition of PDGF-B induction. The second, which inhibits the mitogenic actions of EGF and FGF2, does not require PDGF-B. Regarding the PDGF-B–dependent pathway, src family kinases have been reported to bind to the syndecan-3 cytoplasmic domain, which shares great homology to syndecan-1. Src is an important signaling element for thrombin and PDGF. We have found that the src family kinase inhibitor, PP2, inhibits thrombin-mediated PDGF-B chain induction (data not presented), suggesting the possibility that syndecan-1 may sequester a src family member thus inhibiting PDGF-B chain induction and inhibiting cell proliferation.

Regarding the PDGF-B chain-independent pathway, there are several possible explanations for inhibitory effects of syndecan-1. First, the heparan sulfate glycosaminoglycan chains of syndecan-1 can bind growth factors and can inhibit access of the ligands to receptors. This may be particularly important for the inhibitory effect of syndecan-1 on FGF2-induced SMC proliferation. Relatively low levels of heparan...
sulfate glycosaminoglycans are required for full activation of the FGF receptor by FGF2, but higher levels of the glycosaminoglycans are inhibitory. Of interest, FGF2 activity in bovine SMCs may depend on syndecan-4 and as previously mentioned, maximal thrombin-mediated proliferation of human SMCs requires syndecan-4. These data raise the possibility that syndecan-1 and syndecan-4 play opposing roles in the actions of FGF2.

Like FGF2, the actions of PDGF may also be inhibited by binding to syndecan-1, which may sequester the growth factor. The long form of PDGF-B chain, which has the HS retention motif, binds to HS and chondroitin sulfate glycosaminoglycans with greater affinity than the short form and is less potent for SMC proliferation and migration. The HS retention motif of PDGF-B chain and N-sulfation of the HSPGs are also required for normal pericyte recruitment and localization along microvessels in vivo, presumably because the HS allows the formation of a chemotactic gradient of PDGF. However, pericyte proliferation is not altered by loss of the HS retention motif in PDGF-B chain. Interestingly, thrombin has been shown to cleave the long form of PDGF-B chain and release it from the cell layer, suggesting that thrombin can induce PDGF-B and also make it more available to the PDGFR.

Syndecan-1 may also inhibit growth factor activity through signaling via its intracellular domain. For example, the intracellular variable (V) region of syndecan-1 is necessary for the inhibitory effect of syndecan-1 on laminin-mediated cell migration, cell spreading and actin bundling by fasin.[43]

Another possible mechanism may relate to the involvement of syndecan-1 in lipid raft function. The work of De Dominis and coworkers suggests that PDGF-BB-mediated proliferation requires intact lipid rafts, whereas migration does not. Syndecan-1 has a much greater effect on PDGF-BB-induced SMC proliferation than on migration, and it is known to mediate endocytosis of lipoprotein lipase and lipoproteins via lipid rafts and after which it then recycles back to the cell surface. In addition, Hayashi et al have reported that the small GTPase, Rab5, regulates syndecan-1 shedding and suggest that syndecan-1/Rab5 may be involved in endocytosis of β1 integrin or other receptors.[46,47] These data suggest the possibility that syndecan-1 may regulate PDGFR turnover in lipid rafts. It is of interest that PDGFRs in lipid rafts are more highly phosphorylated and have a longer half-life.[50]

In conclusion, we report that the lack of syndecan-1 leads to robust intimal thickening after carotid injury. The syndecan-1–null mouse used in these studies has a C57Bl/6 background. The C57Bl/6 mouse is relatively unresponsive to carotid ligation injury.[51] Whether syndecan-1 plays a role in the strain-dependency of the response to arterial injury is not clear.

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We thank Jessie Deou for technical assistance and Qinglang Li and William C. Parks for reagents and helpful discussions.

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Disclosures
None.

References


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**Supplement Material for Fukai et al.**

Figure I. Expression of syndecans in serum-starved wild-type and syndecan-1 null SMCs. Data are expressed as fold of wild-type mRNA for syndecan-1 (sdc1), syndecan-2 (sdc2), syndecan-3 (sdc3) and syndecan-4 (sdc4). N=3. *- P<.05 wild-type vs. syndecan-1 null. Inset: dot blot for syndecan-1 protein in cell extracts of wild-type and syndecan-1 null SMCs in duplicate.

Figure II. Morphometric analysis of syndecan-1 null and wild-type carotid arteries 28 days after ligation injury. * P<.05 vs wild-type

Figure III. Proliferation of wild-type and syndecan-1 null SMCs in response to PDGF-BB, FGF2 (A), thrombin, and EGF (B) measured using the MTT assay. Results (mean ± SEM of 3 experiments) are presented relative to the day 1 value of serum alone set to 1. Basal medium alone (1% serum) maintained cell number with no change over 4 days.

Figure IV. The effect of siRNA to syndecan-1 on syndecan-1 mRNA in wild-type SMCs treated with 10% FBS for 28 hours. Results are presented relative to untreated wild-type control ± SEM (n=3). *p<.05 vs. scrambled siRNA.

Figure V. Migration in response to stimulation of wild-type and syndecan-1 null SMCs with either thrombin (10 nM), PDGF-BB (10 ng/ml), or FGF2 (10 ng/ml). † P<.05 PDGF-BB in syndecan-1 null vs. wild-type; N=3 for thrombin and PDGF-BB, N=2 for FGF2.
Figure VI. Effect of siRNA to PDGF-B and PDGFRβ on expression of PDGF-B and PDGFRβ. The effect of siRNA to PDGF-B chain on levels of PDGF-B mRNA 6 hours after growth factor stimulation for DNA synthesis (A) and 4 days after growth factor stimulation for cell proliferation (B). (C) The effect of siRNA to PDGFR β on PDGFRβ protein 28 hours after stimulation with 10% FBS for DNA synthesis. Data are presented relative to wild-type untreated control ± SEM (n=4). *p<.05 vs. scrambled siRNA.

Figure VII. The effect of siRNA to PDGF-B on the proliferation of wild-type and syndecan-1 null SMCs in response to 10% serum, 10 nM thrombin and 10 ng/ml PDGF-BB. Data are presented as the fold of day 1 control values (mean ± SEM of 3-4 experiments) determined using the MTT assay. * P<.05 scrambled control vs. siRNA to PDGF-B.
Figure 1

A bar graph showing the relative expression of mRNA and protein levels in wild-type and Synd-1 null conditions for Sdc1, Sdc2, Sdc3, and Sdc4. The y-axis represents relative expression, with wild-type expression set as 1.0. The x-axis lists the syntenic clusters Sdc1, Sdc2, Sdc3, and Sdc4. The mRNA levels are represented by open bars, and the protein levels by black bars. The diagram also includes an inset image of a protein blot for Synd-1 null conditions.
Figure II

![Bar graph showing area comparison between wild-type and syndecan-1 null for different regions: Lumen, EEL, Intima, Media.](Image)
Figure III
Figure IV

![Bar chart showing Syndecan-1 mRNA (fold of control) for Scrambled and Syndecan-1 treatments](image-url)

- Scrambled: High expression
- Syndecan-1: Low expression with a significant decrease

* denotes statistical significance.
Figure VIA
Figure VIC

![Bar chart showing PDGFRβ levels in Scrambled and PDGFRβ conditions for Wild-type and Syndecan-1 null groups.](image)
Figure VII

The graph shows the relative growth (fold control) of cells treated with different conditions. The x-axis represents different treatments: Control, Serum, PDGF-BB, Thrombin. The y-axis represents the relative growth. The legend indicates the following treatments:
- Non-transfected
- Scrambled siRNA
- PDGF-BB siRNA

The graph is divided into two sections: Wild-type and SDC1 null. The SDC1 null section shows a significant increase in relative growth compared to the Wild-type section for the PDGF-BB treatment. The asterisk (*) denotes a statistically significant difference.
Table I. Forward and reverse primers used for real time reverse transcriptase-polymerase chain reaction (QPCR) of syndecans-1, -2, -3, -4, PDGF-B and ARPP.

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<thead>
<tr>
<th>Primers</th>
<th>Forward</th>
<th>Reverse</th>
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<tr>
<td>Sdc1</td>
<td>ATGGCTCTGGGGATGACTCT</td>
<td>TCCAAGTGGAAGGTGTCTGC</td>
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<td>Sdc2</td>
<td>GTGGTGTGATCGGCTTTTCTC</td>
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<tr>
<td>PDGFB</td>
<td>TGTCCCTGCCTTTTCCACT</td>
<td>GCAGACTGA AGGGCACATGA</td>
</tr>
<tr>
<td>ARPP</td>
<td>TCACTGTGCCAGCTCAGAAC</td>
<td>TCCCACCTTGCTCCAGTCT</td>
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Table II. Attachment of wild-type and syndecan-1 null SMCs.

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<tr>
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<th>Fibronectin</th>
<th>Collagen</th>
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<td></td>
<td>Wild-type</td>
<td>Syndecan-1 null</td>
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<tr>
<td></td>
<td>0.83 ± 0.23</td>
<td>0.94 ± 0.22</td>
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Cells were allowed to attach for 20 minutes to plastic coated with 10 μg/ml fibronectin or 100 μg/ml monomeric type I collagen. Data are expressed as mean OD$_{550}$ ± SEM of 6 experiments.