In vitro differences between venous and arterial-derived smooth muscle cells: potential modulatory role of decorin

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Abstract

Objective: We analyzed the phenotypic and functional differences between venous and arterial smooth muscle cells (SMC) and the role of decorin in modulating these differences.

Methods and results: SMC were isolated from the jugular veins and carotid arteries of male white New Zealand rabbits. Venous SMC demonstrated increased proliferation (2-fold, \( p < 0.001 \)), migration (1.7-fold, \( p < 0.001 \)), and collagen synthesis (4-fold, \( p < 0.001 \)), with decreased adhesion to collagen and fibronectin (1.2-fold, \( p < 0.01 \)) compared to arterial SMC. Higher levels of gelatinase activity (MMP-2 and MMP-9) and tissue inhibitor of metalloproteinase (TIMP) were also observed in venous SMC. Venous SMC demonstrated increased expression of SMemb and decreased expression of SM1—markers of a dedifferentiated and differentiated phenotype, respectively. Arterial SMC produced increased levels of the inhibitory proteoglycan, decorin, compared to venous SMC. Conditioned medium from arterial SMC (ASMC-CM) significantly decreased DNA synthesis, collagen synthesis, and gelatinase activity in venous SMC. Removal of decorin from ASMC-CM by immunoprecipitation significantly reversed the inhibitory effects of ASMC-CM on venous SMC proliferation and collagen synthesis but did not affect gelatinase activities.

Conclusion: Venous SMC are more dedifferentiated and demonstrate increased proliferative and synthetic capacity than arterial SMC. Differential decorin expression between arterial and venous SMC contributes to these differences in biologic behavior. Venous SMC properties may contribute to accelerated atherosclerosis in venous bypass grafts.

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1. Introduction

Coronary artery bypass graft surgery is one of the most frequently performed surgical procedures in the United States, with greater than 500,000 performed in 1999 [1]. Saphenous veins are required in more than 70% of coronary bypass procedures [2]. Approximately 30–50% of venous bypass grafts develop significant narrowing and/or occlusion due to an accelerated form of atherosclerosis within 5–10 years [3].

Vascular smooth muscle cell (SMC) migration, proliferation, and extracellular matrix (ECM) synthesis have been implicated in the pathogenesis of atherosclerosis and vascular repair in both arteries and vein grafts [4,5]. Hemodynamic factors in the arterial circulation involving shear stress and blood pressure have been shown to influence SMC behaviour in “arterialized” vein grafts [6,7]. It is not clear whether differences in biological behavior between venous-derived and arterial-derived SMC may also play an...
important role in atherosclerosis development observed in vein grafts.

The objective of this study was to identify differences in phenotype and function between venous and arterial SMC. Our results indicate that venous SMC were more dedifferentiated, proliferative, migratory, and synthetic than arterial SMC. We also show that venous SMC proliferation and synthetic activity were inhibited by conditioned medium from arterial SMC (ASMC-CM) and by decorin, an inhibitory proteoglycan, which was more highly secreted by arterial SMC. Decorin immunoprecipitation of ASMC-CM reversed its inhibitory effects on venous SMC proliferation and collagen synthesis.

2. Materials and methods

2.1. Specimens

2.1.1. Cell culture

The left jugular vein and carotid artery of male New Zealand white rabbits were dissected free from surrounding tissues, with minimum disruption to the adventitia for in vitro cultures. The protocol used is in accordance with the guidelines set out by the University of Toronto and approved by the St. Michael’s Hospital Animal Care Committee. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). After gentle removal of endothelial cells, SMC were grown in culture as previously described [8]. SMC of passage 2 were cultured in Dulbecco’s modified Eagle’s medium (DMEM; Invitrogen-Life Technologies, Paisley, England, UK) supplemented with 2% penicillin–streptomycin and 10% fetal calf serum (FCS) at 37°C/5% CO2/95% humidity. All experiments were performed in triplicate, and were repeated a minimum of three times from SMC of at least three different animals. In a separate set of experiments, conditioned medium collected from confluent monolayers of arterial SMC (ASMC-CM) or venous SMC (VSMC-CM) was used.

2.2. Differentiation markers

The differentiation status of venous and arterial SMC was determined by immunofluorescence staining for smooth muscle myosin heavy chain embryonic (SMemb) and smooth muscle myosin heavy chain-1 (SM1) cytoplasmic proteins to identify the dedifferentiated “synthetic” or differentiated “quiescent” phenotypes, respectively [9–11]. Briefly, cells (10^3/chamber) were seeded onto eight-chamber culture slides (Costar) and grown to about 60% confluency. Cells were then fixed using 4% paraformaldehyde for 15 min and nonspecific binding was blocked with 1% BSA and 5% normal goat serum for 30 min. Mouse monoclonal primary antibodies directed against SMemb or SM1 (both at 1:3000 dilution; Seikagaku America, Cape Cod, USA) were added and incubated at 4°C overnight. Goat antimouse Cy3-labeled secondary antibody was then added followed by incubation with smooth muscle α-actin (1:250 dilution, FITC-conjugated; Sigma-Aldrich, St. Louis, MO, USA). All slides were counterstained with 4,6-diamidino-2-phenylindole (1 μg/ml). The differentiation status of the cells was also confirmed by Western blotting using mouse anti-SMemb, SM1 (both at 1:2000; Seikagaku America), and alpha-actin (1:1000; Dako) primary antibodies. Antimouse IgG-HRT was used for detection of primary antibodies and revealed using chemiluminescence detection system (Sigma, St. Louis, MO, USA) followed by autoradiography using BioMax film (Kodak, Rochester, NY, USA).

2.3. Growth assays

DNA synthesis was measured in DMEM containing 10% FCS using the [3H]thymidine (Amersham, Upsalla, Sweden) incorporation method as previously described [8,12]. Briefly, 10^4 cells/well were seeded onto 24-well tissue culture plates and allowed to adhere for 24 h. [3H]thymidine (1 μCi/ml; Amersham Biosciences, Piscataway, NJ, USA) was then added to the cells and incubated at 37°C for 24 h. Cells were then washed, fixed with 10% trichloroacetic acid (TCA), and lysed with 0.3 N NaOH and 1% SDS solution, and radioactivity was measured using a Beckman liquid scintillation counter. An aliquot of the cell extract was used for protein measurements (De Protein Analysis; BioRad Laboratories, Hercules, CA, USA). For analysis of the effects of ASMC-CM on venous SMC DNA synthesis, venous SMC were pretreated with ASCMC-CM for 24 h and [3H]thymidine (1 μCi/ml) was added and incubated further for 24 h.

Cell proliferation was determined using a colorimetric assay as previously described [13]. Briefly, 5×10^3 cells/well were seeded in 96-well plates and cell proliferation was measured daily for a period of 8 days. The cells were fixed with 4% paraformaldehyde, stained with 0.5% toluidine blue in 4% paraformaldehyde, and then solubilized in 100 μl of 1% SDS. Absorbance was measured in a microplate reader (Molecular Devices).

2.4. Adhesion assay

SMC adhesion was determined using a previously described method [13]. Type I collagen Vitrogen 100 (100 μg/ml; Collagen Biomaterials) or fibronectin (100 nM; Sigma) was coated onto the surface of 96-well plates overnight at 4°C. The wells were washed and nonspecific binding was blocked with 1% BSA at 37°C for 1 h. Cells (3×10^3 cells/well) were then added to the plates and allowed to attach at 37°C for 90 min. Nonadherent cells were washed off with PBS and the remaining attached cells
were fixed with 4% paraformaldehyde and stained with 0.5% toluidine blue. Absorbance was measured in a microplate reader (Molecular Devices).

2.5. Migration assay

SMC migration through a collagen-coated membrane was determined using a modification of a previously described method [8]. Neuro Probe 48-well microchemotaxis chambers (Costar) with PVP-free polycarbonate filter (8.0 μm pore size) were coated with 100 μg/ml Type I collagen (Vitrogen 100) for 6 h in a humidified chamber. The bottom well of the Boyden chamber was then filled with 250 μl of serum-free medium containing PDGF-BB (10 ng/ml; R&D Systems, Minnesota, USA). Quiescent cells were suspended in serum-free medium and added to the upper well of the microchemotaxis chamber and incubated at 37°C for 24 h. To identify random migration, cells were allowed to move towards a serum-free medium without PDGF. Migrated cells to the lower side of the membrane were fixed and stained for cytoplasmic and nuclear structures (Diff-Quick staining kit; VWR). The membranes were then mounted on glass slides and migrated cells were counted using a conventional light microscope under 100× magnification. The results were expressed as number of migrated cells per high-power field (HPF). Five random fields were counted per membrane.

2.6. Gelatin zymography

Conditioned medium was collected from confluent cells that were incubated in serum-free medium for 72 h. Aliquots corresponding to 30 μg of cell protein were loaded onto gels containing 0.1% gelatin substrate (Invitrogen-Life Technologies) and subjected to electrophoresis as previously described [14]. Gelatinolytic activities were quantified by densitometric analysis (BioRad Gel 1000 documentation system and Molecular Analyst software). For positive control, rabbit aortic SMC were stimulated with phorbol 12-myristate 13-acetate (50 ng/ml) for 72 h and conditioned medium was used.

In a separate experiment, confluent monolayer of venous SMC were treated with ASMC-CM for 72 h and then analyzed for gelatin zymography.

2.7. Tissue inhibitor of metalloproteinase (TIMP) reverse zymography

TIMP activity was determined by reverse zymography using a method previously described [15]. Aliquots of conditioned medium corresponding to 30 μg of cell protein were subjected to electrophoresis on gels containing gelatin substrate (0.1% wt/vol) and MMP-2 (0.13 μg/ml; Chemicon International, Temecula, USA). The gels were then washed with 2.5% Triton X-100 three times for 20 min each and incubated in developing buffer (50 mmol/l Tris base, 15 mmol/l CaCl₂) overnight at 37°C. The gels were then fixed in 10% TCA, stained with Coomassie blue (Sigma) for 30 min, and destained for 3 days. TIMPs were identified as undigested bands remaining against a clear background.

2.8. Collagen synthesis assay

Confluent cells in six-well plates were serum-starved for 24 h and DMEM containing FCS (2%), ascorbic acid (50 μg/ml; Sigma), and [¹⁴C]proline (2.5 μCi/ml; Amersham) was added and incubated at 37 °C for 6 h. Collagen synthesis was determined in the culture medium using a bacterial collagenase digestion method as previously described [8,16].

2.9. PDGF, TGF-β1, and decorin secretion in SMC conditioned medium

To identify the factors involved in regulating SMC function, the conditioned medium was assessed for various protein expressions. Confluent cells were washed three times with PBS and incubated in both serum-stimulated (10% FCS) and serum-free medium for 24 h. Conditioned medium was collected, mixed with an equal volume of 20% TCA, and kept at 4°C overnight to precipitate proteins. The precipitate was washed three times with 100% ethanol and dissolved in warm (65°C) SDS sample loading buffer. Aliquots corresponding to 30 μg of cell protein were analyzed by 4–20% SDS-PAGE and electrotransferred onto nitrocellulose membranes. Membranes were immunoblotted with antidecorin monoclonal antibody 6D6 (generous gift from Dr. Paul G. Scott, University of Alberta, Edmonton, Alberta, Canada), goat anti-PDGF antibody (R&D Systems), and anti-TGF-β1 monoclonal antibody (R&D Systems). Antimouse IgG-HRP or antigoat IgG-HRP secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, USA) were used for detection of primary antibodies and revealed using chemiluminescence detection system followed by autoradiography using BioMax film as described above. Equal loading of proteins was determined by staining of the membrane with Ponceau S (Sigma).

2.10. Effect of decorin on venous SMC DNA synthesis

Venous SMC were pretreated for 24 h with either VSMC-CM, VSMC-CM with exogenous purified decorin (20 μg/ml; Sigma), ASMC-CM, decorin-immunoprecipitated ASMC-CM (see below), or IgG-immunoprecipitated ASMC-CM (see below). DNA synthesis was determined 24 h after addition of 1 μCi/ml [³H]thymidine.

2.11. Effect of decorin on venous SMC collagen synthesis

Venous SMC were treated for 24 h with either VSMC-CM, VSMC-CM with exogenous purified decorin (20 μg/ml; Sigma), ASMC-CM, decorin-immunoprecipitated ASMC-CM (see below), or IgG-immunoprecipitated ASMC-CM.
Collagen synthesis was then determined in 2% FCS culture medium supplemented with 50 μg/ml ascorbic acid and 2.5 μCi/ml [14C]proline for 6 h.

2.12. Effect of decorin on gelatinase activity

Venous SMC were treated for 24 h with either VSMC-CM, VSMC-CM with exogenous purified decorin (20 μg/ml; Sigma), ASMC-CM, decorin-immunoprecipitated ASMC-CM (see below), or IgG-immunoprecipitated ASMC-CM (see below) for 72 h and assessed for gelatinase activity.

2.13. Decorin immunoprecipitation

ASMC-CM was incubated at 4 °C for 6 h with 2 μg of antidecorin antibody. The decorin antibody complex was removed by adhering to protein G Sepharose (Pharmacia Biotech) beads at 4 °C overnight followed by centrifugation. The absence of decorin in ASMC-CM after immunoprecipitation was confirmed by Western blotting (data not shown). Nonspecific IgG-immunoprecipitated ASMC-CM with nonimmune mouse IgG (Sigma) served as a control for these studies.

2.14. Statistical analysis

All measurements are expressed as mean ± standard error of mean. Statistical comparisons were done by unpaired Student’s t tests. Statistical significance was defined as p < 0.05.

3. Results

3.1. SMC phenotype

Venous SMC were smaller, more spindle-shaped, and expressed less α-actin than arterial SMC (Fig. 1A). In serum-free conditions, both venous and arterial SMC expressed SM1 (data not shown) and α-actin, indicating a differentiated or quiescent phenotype. In serum-stimulated conditions, venous SMC demonstrated a more dedifferentiated phenotype with increased SMemb expression and decreased α-actin expression (Fig. 1A, III) compared to arterial SMC (Fig. 1A, IV). Western blot analysis confirmed higher levels of SMemb and lower levels of SM1 in serum-stimulated venous SMC (Fig. 1B).

3.2. Cell growth

DNA synthesis was 2-fold higher in venous SMC 48 h after seeding (Fig. 2A). Cell proliferation was significantly higher in venous SMC during the log phase (days 4–7) with cell density nearly 2-fold higher at the plateau phase (day 8) compared to arterial SMC (Fig. 2B).

3.3. Cell adhesion and migration

Adhesion to both collagen and fibronectin was significantly lower for venous SMC compared to arterial SMC (Fig. 3A). PDGF-induced migration of venous SMC was 1.7-fold higher than arterial SMC (Fig. 3B).

Fig. 1. (A) Immunostaining for α-actin and SMemb. Subconfluent SMC were stained with anti-SMemb (red) and anti-α-actin (green) and counterstained with DAPI (blue). Serum-deprived venous and arterial SMC did not express SMemb (I and II, respectively), indicating a differentiated phenotype. Serum-stimulated venous SMC expressed more SMemb and less α-actin (III) compared to arterial SMC (IV), indicating a more dedifferentiated phenotype. (B) Western blotting for SMemb and SM1. SMemb levels were higher in venous SMC while SM1 levels were higher in arterial SMC (B).
3.4. Gelatinase and TIMP activities

Venous SMC demonstrated a faint 92-kDa gelatinase band, corresponding to MMP-9, which was not evident in arterial SMC (Fig. 4A). A more prominent 68-kDa gelatinase band, corresponding to active MMP-2, was also observed in venous SMC (Fig. 4A). Densitometric analysis demonstrated significantly higher active MMP-2 ($p<0.05$) and MMP-9 ($p<0.01$) activity in venous SMC compared to arterial SMC (data not shown). Treatment of venous SMC with ASMC-CM markedly reduced MMP-9 and MMP-2 activity (Fig. 4B).

Reverse zymography showed that TIMP-1 and TIMP-2 levels were higher in venous SMC compared to arterial SMC (Fig. 4C).

3.5. Collagen synthesis

Collagen synthesis was fourfold higher in venous SMC compared to arterial SMC (Fig. 5A). Treatment of venous SMC with ASMC-CM resulted in a significant reduction in collagen synthesis (Fig. 5B).

3.6. Decorin, PDGF, and TGF-β1 expression

There were higher levels of decorin, PDGF, and TGF-β1 protein in arterial SMC compared to venous SMC in both serum-stimulated (Fig. 6A) and serum-free conditions (data not shown).

3.7. Effect of decorin immunoprecipitation on PDGF and TGF-β1 levels in venous SMC

As expected, decorin level was decreased in decorin-immunoprecipitated ASMC-CM, while IgG immunoprecipitation had no effect (Fig. 6B). PDGF and TGF-β1 protein expression was decreased in venous SMC treated with decorin-immunoprecipitated ASMC-CM compared to control ASMC-CM and IgG-immunoprecipitated ASMC-CM (Fig. 6B).

3.8. Effect of decorin on venous SMC behaviour

DNA and collagen synthesis (Fig. 7A and B, respectively) were significantly reduced in venous SMC treated with either ASMC-CM or exogenous decorin. Treatment of venous SMC with decorin-immunoprecipitated ASMC-CM, but not IgG-immunoprecipitated ASMC-CM, significantly reversed the inhibitory effects of ASMC on venous SMC DNA and collagen synthesis (Fig. 7A and B, respectively).

Gelatinase activity was also reduced in venous SMC treated with ASMC-CM compared to VSMC-CM (Fig. 4B). However, there were no differences between decorin-immunoprecipitated ASMC-CM or IgG-immunoprecipitated ASMC-CM (data not shown).

4. Discussion

This study is the first detailed in vitro comparison of venous and arterial SMC. The major finding of this study is
that venous SMC demonstrate increased proliferation, migration, and synthetic capacity compared to arterial SMC in serum-stimulated culture conditions. This is supported by increased expression of the dedifferentiation marker, SMemb, and decreased SM1 and α-actin expression in venous SMC. A similar pattern of venous SMC dedifferentiation has been described in the initial few

Fig. 4. (A) Representative gelatin zymogram. Active MMP-2 (68 kDa) and latent MMP-9 (92 kDa) were increased in venous SMC (lanes 1–4) compared to arterial SMC (lanes 5–8). For positive controls, aortic SMC were stimulated with phorbol 12-myristate 13-acetate (50 ng/ml), which induces production and activation of MMP-2 and MMP-9. (B) Treatment of venous SMC with ASMC-CM markedly reduced MMP-9 and MMP-2 activity (lanes 3 and 4). (C) Representative reverse zymogram. There were increased levels of TIMP-1 (28 kDa) and TIMP-2 (24 kDa) in venous SMC (lanes 1 and 2) compared to arterial SMC (lanes 3 and 4). ASMC-CM, conditioned medium from arterial SMC.

Fig. 5. (A) Collagen synthesis was significantly higher in venous SMC. (B) Venous SMC treated with ASMC-CM demonstrated a marked reduction in collagen synthesis. *p<0.001 compared to arterial SMC; †p<0.001 compared to VSMC-CM.

Fig. 6. (A) Western blot analysis of conditioned medium from venous (lanes 1 and 2) and arterial (lanes 3 and 4) SMC. Western blots were probed with antibody against decorin, PDGF-BB, and TGF-β1. Decorin, PDGF-BB, and TGF-β1 levels were higher in the ASMC-CM. (B) Western blot analysis of decorin, PDGF-BB, and TGF-β1 levels in ASMC-CM (lane 3). Decorin, PDGF, and TGF-β1 levels were markedly reduced in the decorin-immunoprecipitated ASMC-CM (lane 2) compared to control ASMC-CM and nonimmune IgG-treated ASMC-CM.
months after vein graft construction in neointimal SMC of
vein grafts [10,11]. Therefore, cultured venous SMC appear
to be a suitable model to study early stages of vein graft
repair.

The differentiation state of SMC has been implicated in
various vascular pathologies such as atherosclerosis [9,17]
and intimal hyperplasia [10,11]. The dedifferentiated phe-
notype for venous SMC reported in this study is consistent
with the cellular behavior characterized by increased
proliferation, migration, and synthetic capabilities. Our
results show increased levels of collagen synthesis and
gelatinase activity in venous SMC. Collagen synthesis and
accumulation are integral aspects of vascular repair [14,18].
Previous studies have also shown that gelatinase activity is
a critical component of SMC migration in vein graft disease
[19] and the formation of intimal hyperplasia after arterial
injury and in vein grafts [14,20,21].

A second important observation of this study is that
ASMC-MC has an inhibitory effect on venous SMC
conditioned medium for regulatory proteins. PDGF and
TGF-β1, are important growth factors involved in SMC
growth and matrix synthesis [22,23]. Decorin is a small
leucine-rich chondroitin/dermatan sulfate proteoglycan of
about 100 kDa in size with a 40-kDa core protein. It serves
as a linker ligand that attaches two parallel neighbouring
collagen molecules, helping to stabilize the collagen fibrils
[24]. We and others have shown that decorin can also bind
to PDGF [8] and TGF-β [12,25] and is a potent inhibitor of
these growth factors on SMC activities such as cell
attachment, migration, proliferation, and collagen synthesis.
In this study, we show that despite higher levels of PDGF
and TGF-β1 in arterial SMC-conditioned medium, these
cells proliferated at a lower rate (based on thymidine
incorporation to assess DNA synthesis) and synthesized
less collagen than venous-derived SMC. Our data suggest
that these cellular effects in arterial SMC may be due to
increased synthesis of the inhibitory proteoglycan, decorin.
The increased expression of decorin in the ASMC-CM may
explain the inhibitory effects of this medium on venous
SMC DNA synthesis and collagen synthesis since signifi-

![Fig. 7. (A) Effect of decorin on venous SMC DNA synthesis. DNA synthesis was significantly reduced in venous SMC treated with either VSMC-CM with
exogenous decorin or ASMC-CM compared to control VSMC-CM. Decorin-immunoprecipitated ASMC-CM, but not IgG-immunoprecipitated ASMC-CM,
significantly reversed the inhibitory effects of ASMC. *p<0.001 compared to control VSMC-CM; †p<0.001 compared to control ASMC-CM or IgG-
immunoprecipitated ASMC-CM. (B) Effect of decorin on venous SMC collagen synthesis. Venous SMC treated with exogenous decorin or ASMC-CM
resulted in a significant reduction in collagen synthesis. Venous SMC treated with decorin-immunoprecipitated—but not control IgG-immunoprecipitated—
ASMC-CM exhibited a significant restoration of collagen synthesis. *p<0.001 compared to VSMC-CM; †p<0.001 compared to control ASMC-CM or IgG-
immunoprecipitated ASMC-CM. VSMC-CM, conditioned medium from venous smooth muscle cells; ASMC-CM, conditioned medium from arterial smooth
muscle cells.]
cant increases in DNA synthesis and collagen synthesis were restored in venous SMC treated with ASMC-CM in which decorin was removed by immunoprecipitation.

We also found that venous SMC treated with decorin-immunoprecipitated ASMC-CM produced lower protein levels of PDGF and TGF-β compared to ASMC-CM and IgG-immunoprecipitated ASMC-CM. This reduction in overall PDGF and TGF-β levels is attributed to removal of decorin-bound growth factor by the decorin immunoprecipitation. However, unbound PDGF and TGF-β are still present in the ASMC-CM, which has an overall stimulatory effect on SMC proliferation and collagen synthesis when there is no decorin present. Therefore, the predominant effect of decorin immunoprecipitation is the unopposed stimulatory effects of PDGF and TGF-β, despite a reduction in the overall PDGF and TGF-β protein levels. These findings are consistent with previous observations that decorin overexpression results in increased growth factor (PDGF and TGF-β) binding to decorin, which neutralizes the growth-stimulatory effect of these growth factors [8,25,26]. In experimental vein graft studies, decorin has been identified in the hypocellular regions of the neointima, suggesting an inhibitory in vivo effect of decorin on SMC proliferation and intimal hyperplasia [11]. In addition, we have recently shown that decorin overexpression after arterial injury reduced intimal hyperplasia and collagen accumulation [8].

Although ASMC-CM decreased both MMP-9 and MMP-2 activities, there were no differences in gelatinase activity in venous SMC treated with either decorin-immunoprecipitated or control IgG-immunoprecipitated ASMC-CM. This finding suggests that there are additional unidentified inhibitory secretory factors in ASMC-CM that regulate gelatinase activity. Several proteoglycans other than decorin have been shown to modulate SMC behaviour. Heparan sulphate proteoglycans (HSPG) such as perlecan and syndecan are possible candidates since their core protein can bind to a variety of growth factors including FGF [27], VEGF [28], PDGF [29], and TGF-β [30]. HSPGs such as perlecan have been shown to inhibit entry of cells into the S-phase of the cell cycle, thereby preventing cellular proliferation [31], as well as promote the formation of cellular adhesion sites, thereby inhibiting cellular migration [26]. Future studies will need to delineate the exact mechanism by which additional inhibitory factors, such as these proteoglycans, may be modulating differences in venous and arterial SMC behaviour.

There are a few reports of differences between arterial and venous SMC in the literature. Yang et al. [32] have also shown enhanced proliferation of venous compared to arterial SMC in response to exogenous PDGF-BB stimulation, despite similar PDGF α- and β-receptor expression and activation. Coculture studies of endothelial and SMC have shown that differences between arterial- and venous-derived endothelial cells may affect SMC behavior. In coculture conditions, venous endothelial cells induce greater proliferative responses in both arterial and venous SMC than arterial endothelial cells [33]. Thus, “cross-talk” between endothelial cells and SMC may also regulate SMC behavior [34].

4.1. Clinical relevance

Although the saphenous vein remains a commonly utilized conduit in coronary artery bypass grafting, it is often plagued by vein graft diseases such as fibrointimal hyperplasia and accelerated atherosclerosis within 5–10 years after surgery [3]. The causes for vein graft diseases are multifold. Studies have shown that many factors such as surgical trauma [35] and hemodynamic changes [36,37] are important in the development of vein graft intimal hyperplasia. Furthermore, consistent with our in vitro results, venous SMC dedifferentiation and proliferation have been shown in vein graft intimal hyperplasia [10,11]. Based on our study, venous SMC have particular intrinsic characteristics that may contribute to the development of vein graft disease. Our data also suggest that strategies using inhibitory proteoglycans, such as decorin, may favorably alter venous SMC behavior contributing to vein graft intimal hyperplasia.

A limitation in our in vitro culture model is that our analysis is based on rabbit SMC that originate from carotid arteries and jugular veins. It is possible that SMC behavior may vary in different vascular beds and between species. Future studies will need to address these issues by using human SMC from the saphenous vein and coronary artery to increase the generality of our findings.

In conclusion, we have shown that cultured venous and arterial SMC demonstrate differences in phenotype, function, and ECM protein synthesis. Our data also suggest that differences in secretory factors, such as decorin, may be contributing to the altered SMC behaviour. These differential phenotypic and functional characteristics of venous SMC evident in culture conditions may account for the accelerated form of atherosclerosis that occurs in veins when they are grafted into the arterial circulation during coronary artery bypass surgery. Therefore, decorin overexpression in vein grafts may be an important therapeutic strategy to prevent vein graft disease.

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References


