Inhibition of Versican Synthesis by Antisense Alters Smooth Muscle Cell Phenotype and Induces Elastic Fiber Formation In Vitro and in Neointima After Vessel Injury

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Abstract—The proteoglycan versican is implicated in several atherogenic events, including stimulation of vascular smooth muscle cell (VSMC) growth and migration, retention of lipoproteins, and promotion of thrombogenesis. A high content of intimal versican also correlates with a low content of elastin, suggesting an inhibitory role for versican in elastogenesis. To determine whether reduced production of versican can be used to enhance elastogenesis, we transduced Fischer rat VSMC (FRSMC) with a versican antisense sequence using the retroviral vector LXSN. Stable expression of versican antisense (LVaSN) significantly reduced versican production, induced a flattened morphology, reduced cell proliferation and migration, increased tropoelastin synthesis, increased elastin binding protein (S-Gal/EBP), and increased deposition of elastic fibers in long-term cultures. Add-back of chondroitin sulfate chains, or versican, decreased S-Gal/EBP and elastic fiber formation. LVaSN cells seeded into balloon catheter-injured rat carotid arteries formed neointimae containing low levels versican, increased amounts of S-Gal/EBP, and increased elastin deposits 7 days postinjury. At 4 weeks, neointimae formed from LVaSN cells were highly structured and contained multiple layers of elastic fibers and lamellae. These results indicate a central role for versican and its constituent chondroitin sulfate chains in controlling cell phenotype, elastogenesis, and intimal structure. (Circ. Res. 2006;98:0-0.)

Key Words: versican antisense | elastogenesis | vascular injury | remodeling

Versican, a chondroitin sulfate (CS) proteoglycan, is a major determinant for the structural and physiological properties of arterial wall extracellular matrix.1,2 The aminoterminal globular domain (G1) of versican core protein binds to hyaluronan (HA) and affects cell adhesion, proliferation, and migration.3-5 The carboxy-terminal domain (G3) mediates changes in morphology and adhesiveness by interacting with various other matrix molecules such as tenascin-R, fibrillin-1, and fibulins.6-9 Flanked between G1 and G3 is the glycosaminoglycan (GAG) binding region, which is encoded by 2 exons (α and β), which may be differentially spliced to give rise to 4 versican variants: V0 (α and β exons), V1 (β exon only), V2 (α exon only), and V3 (neither exon).10

These GAG subdomains, in addition to conferring tissues with viscoelastic properties, can differentially affect growth and apoptosis11 and interact with other extracellular matrix components.12 Furthermore, CS inhibits assembly of elastic fibers, a process controlled by the 67-kDa cell-surface elastin components.12 Furthermore, CS inhibits assembly of elastic fibers, a process controlled by the 67-kDa cell-surface elastin components.12 Furthermore, CS inhibits assembly of elastic fibers, a process controlled by the 67-kDa cell-surface elastin components.12 Furthermore, CS inhibits assembly of elastic fibers, a process controlled by the 67-kDa cell-surface elastin components.12 Furthermore, CS inhibits assembly of elastic fibers, a process controlled by the 67-kDa cell-surface elastin components.12 Furthermore, CS inhibits assembly of elastic fibers, a process controlled by the 67-kDa cell-surface elastin components.12 Furthermore, CS inhibits assembly of elastic fibers, a process 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into balloon-injured artery. The results support a central role for CS-containing versican in controlling cell phenotype, elastogenesis, and intimal structure.

Materials and Methods

Cell Culture

Aortic smooth muscle cells from male Fischer 344 rats (FRSMC) (Simenson Co, Gilroy, Calif) were obtained and cultured as described previously. Cells between 3 and 10 passages were used for the experiments.

Retroviral Transduction

Full-length antisense and sense V3 sequences were used to construct retroviral vectors using methodology described previously for V3, \(^{21}\) Briefly, rat V3 cDNA and the complementary product were each inserted into the \textit{BamHI} site of empty retroviral vector (LXSN) to produce V3 and versican antisense-containing vectors (LV3SN and LVSaSN), respectively. Orientation of the inserts was confirmed by PCR. LXSN, LV3SN, and LVaSN retroviruses, produced by transfection of PA317 packaging cells, were used to transduce cultured FRSMC as described previously. \(^{22–24}\) Three LXSN, 1 LV3SN, and 3 LVaSN FRSMC clones between 3 and 10 passages after initial transductions were used for the following experiments.

Detection of Versican Antisense

RT-PCR was performed with ImProm-II Reverse Transcription System (Promega A3800) in accordance with the instructions of the manufacturer. Total RNA from 48-hour cultures of LXSN, LV3SN, and clones 1 to 3 of LVaSN was reverse transcribed with forward primer LXSNF (5’-CCTGAAACCTCTCGTGCA-3’) and reverse primer JL27 (5’-GACTATGGCTGGCACAA-3’).

Versican and Tropoelastin mRNA Levels

Total RNA was isolated and Northern hybridization performed using a pool of V3, versican antisense, and \(\beta\)-gag (V25) probes as described previously. \(^{25}\) Denatured double-stranded V3 cDNA served to detect both sense and antisense versican sequences, whereas V25 identified versicans V0 and V1. Tropoelastin mRNA was detected by a human probe generously provided by Dr C. D. Boyd (University of Hawaii). \(^{26}\)

Versican Production

Cells were seeded at a density of \(5 \times 10^5\) cells/100-mm culture dish, maintained in 10 mL of 10% FBS growth medium for 24 hours, starved for 24 hours in 10 mL of growth medium with 0.1% FBS, and incubated in 10 mL of 10% growth medium containing 100 \(\mu\)Ci/mL \(^{35}\)S-sulfate for a further 24 hours. Labeled medium proteoglycans were isolated as described previously \(^{27}\) and prepared for SDS-PAGE. \(^{28}\) Samples, including a parallel set digested with chondroitin ABC lyase (ICN, Costa Mesa, Calif), were run on a 4% to 12% gradient polyacrylamide-SDS resolving gel with a 3.5% polyacrylamide stacking gel. Loading volumes reflected the amount of proteoglycan produced by an equal number of cells. The gel was processed as described previously, \(^{29}\) and versican production determined from band intensities of undigested and chondroitin ABC lyase digested samples.

Versican Core Proteins

Core proteins of chondroitin ABC lyase-digested medium samples were separated on SDS-PAGE and Western blotted as described previously. \(^{28}\) V0 and V1 core proteins were detected with versican antibody LF99 (rabbit anti-human, kindly provided by Dr Larry Fisher, National Institute of Dental and Craniofacial Research, NIH, Bethesda, Md), diluted 1:5000 in 5 mL of Tris-buffered saline with 2% FBS. \(^{29}\) Following exposure to Kodak XAR-2 film, band intensities were analyzed by NIH Image.

Growth and Migration

Cell growth and migration rates were determined as described previously. \(^{21}\) Migration was determined by measurement of movement of cells into a cell-free zone created by scraping confluent cultures.

Immunodetection of Versican, CS, Tropoelastin, and S-Gal/EBP

Versican

Cells seeded at 5000 cells/well on chamber glass slides were cultured in standard medium for 24 hours, fixed in 0.9% formaldehyde, washed in PBS, incubated for 1 hour with primary versican antibody LF99, washed in PBS, and incubated for 1 hour in Texas Red-conjugated goat anti-rabbit secondary antibody (1:100; Invitrogen T2767). Slides were rinsed in PBS and mounted with Dako Fluorescent Mounting Medium (S3023). Fluorescent micrographs were converted to gray scale in Adobe Photoshop 7.0 and staining intensities measured by NIH Image analysis and expressed as optical density (OD).

Chondroitin Sulfate

Cells cultured for 7 days were fixed for 30 minutes in cold 100% ethanol and immunostained with 5 \(\mu\)g/mL of a monoclonal antibody to CS (C-8035, Sigma) and a fluorescein-conjugated secondary antibody (FITC). \(^{19}\)

Tropoelastin

Seven-day confluent cultures were fixed for 30 minutes in cold 100% ethanol, immunostained with 10 \(\mu\)g/mL polyclonal bovine tropoelastin antibody (Elastin Products Co Inc, Owensville, Mo), and detected by FITC. Three-week cultures, with and without daily CS add-back (200 \(\mu\)g/mL), were fixed in 0.9% formaldehyde and immunostained with the tropoelastin antibody and Texas Red-conjugated secondary antibody.

S-Gal/EBP

Two-day subconfluent cultures were immunostained with 20 \(\mu\)g/mL anti-S-Gal that recognizes the sequence that binds elastin, \(^{13}\) and with anti-EBP (Elastin Products Co Inc), using FITC and Texas Red secondary antibody detection respectively. The latter antibody was also used to assess the effect of 24 hour add-back of versican (200 \(\mu\)g/mL) on EBP staining intensity, which was quantified by NIH Image analysis.

S-Gal/EBP and B-Gal–related proteins were also isolated from cell layers and detected by Western blotting with 3 different antibodies, anti-P-Gal, raised to the \(\beta\)-Gal precursor and recognizes all forms of \(\beta\)-Gal, \(^{13}\) anti-S-Gal, and anti-EBP, as previously described. \(^{15}\) Horseradish peroxidase secondary antibody signals were detected by enhanced chemiluminescence.

Effect of Versican and CS Add-Back on S-Gal/EBP and Elastin Deposition

Cells were seeded at 5000 cells/well on 8-well Laboratory-Tek Chamber Glass Slides (NUNC 177399) and maintained in medium containing 200 \(\mu\)g/mL versican \(^{30}\) or chondroitin sulfate A (Sigma C-8529). The concentration was predetermined by a dose-range experiment of CS (10, 100, 200, and 400 \(\mu\)g/mL) to achieve optimal effect (results not shown). Cells cultured for 24 hours in the versican-containing medium were immunostained with anti-EBP. Cells cultured for 3 weeks in CS-containing medium were immunostained for tropoelastin. Controls were maintained in medium only. Slides were fixed in 0.9% formaldehyde for 10 minutes for the immunocytochemistry. For Western blot detection of P-Gal, S-Gal, and EBP, cells were cultured for 7 days with and without CS (400 \(\mu\)g/mL).

Insoluble Elastin

Quadruple cultures of LXSN and LVaSN (plated at \(5 \times 10^4\) cells/well) were grown to confluence in the 6-well culture dishes. Twenty micromoles of \([\text{H}]\)-valine (New England Nuclear, Boston, Mass)
were added to each well at day 4 along with fresh media, and cells cultured for a further 3 days. At day 7 media were removed and the cell layers scraped in 0.1N NaOH, sedimented by centrifugation, and boiled in 0.5 mL of 0.1N NaOH for 45 minutes to solubilize all matrix components except elastin. The resulting pellets containing the insoluble elastin were solubilized by boiling in 200 µL of 5.7 N HCl for 1 hour and aliquots mixed with scintillation fluid and counted.15

**Balloon Catheter Injury and Cell Seeding of Rat Carotid Arteries**

Balloon injury and cell seeding in Fischer 344 rats were performed in accordance with the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication, revised 1985) as described previously.19,31 Control or antisense-expressing cells (3.75×10^6) were suspended in 150 µL of serum-free growth medium and injected into each balloonated and isolated common carotid artery. Rats were inverted for 2 minutes and then returned to the original position for 13 minutes to allow for even-cell settlement. Following restoration of blood flow and wound closure, rats were maintained on a normal diet and euthanized on days 7 (6 rats from each control and antisense group) and 28 (8 rats from each control and antisense group) as described previously.19 A 50-mg tablet of bromodeoxyuridine (BrdUrd) was subcutaneously implanted in each rat 24 hours before euthanasia for the purpose of measuring cell proliferation.

**Histochemistry and Immunohistochemistry on Seeded Vessels**

Seeded vessels were harvested, fixed in 4% paraformaldehyde, and processed for paraffin embedding as described previously.19 Fivemicrometer sections were stained with Toluidine Blue or orcein to display general tissue morphology and elastin organization. In addition, sections were immunostained for versican or EBP using DAKO Envision rabbit anti-human horseradish peroxidase (DAB).

**TEM and Morphometric Analyses**

Confluent cultures and segments of the seeded carotid arteries were fixed in 3% glutaraldehyde, postfixed in 1% OsO4, stained in 2% uranyl acetate, and embedded in Epon as described previously.32 Sections were mounted on formvar-coated grids (Pro Sci Tech Gcu300), stained in 2% uranyl acetate, and embedded in Epon as described previously.32. Sections were mounted on formvar-coated grids (Pro Sci Tech Gcu300), stained in 2% uranyl acetate, and viewed on a JEOL 1200 EXII microscope. Volume fractions for cell, elastin, collagen, and matrix space were determined by point counting as described previously.19

**Statistics**

Data were analyzed by Student’s t test and ANOVA, and a value of $P<0.05$ was taken as significant.

**Results**

**Expression of Versican Antisense**

LVaSN clones 1 to 3 expressed the predicted 973-bp product of RT-PCR, with clone 2 showing the highest expression. Expression level, however, did not correlate with the degree of versican knockdown or other changes as presented below; all clones were effective at reducing versican. Neither LXSN nor LV3SN cells expressed detectable levels of antisense (Figure 1A).

**Versican Antisense Reduced Versican mRNA and Versican Production**

Total RNA extractions from 14-day cultures of LXSN, LV3SN, and LVaSN were probed for versican expression (Figure 1B). LVaSN cells were negative for all versican transcripts. Similar to previous results,21 LXSN and LV3SN cells showed low levels of V0 at 13 to 11 kb, V1 at 9 to 7.7 kb, and in V3, V3/neo at 6 kb. The level of V1 mRNA in LV3SN cells was increased compared with LXSN. A previ-
ous study, however, showed V1 mRNA levels in LV3SN to be variable.21

[35S]-Labeled versican secreted by LXSN, LV3SN, and LVaSN cells was separated on SDS-PAGE as previously described.27,28 Versican production, measured as the difference in band densities of chondroitin ABC lyase digested and nondigested samples, was reduced by 95% (P<0.001) in the 3 LVaSN clones (Figure 1C). Versican V1 and V0 core proteins, analyzed by Western hybridization, showed a significant reduction of 85% (P<0.005) in band density of the LVaSN clones compared with LXSN clones (Figure 1D).

Low-density cultures of LVaSN cells showed reduced immunostaining for versican compared with LXSN cells (Figure 1E). The mean percentage decrease in staining intensity, calculated from OD measurements for 4 independent experiments, was 53.3±2.3% (P<0.001). Similarly, confluent 7-day cultures of LVaSN cells stained less intensely for CS than LXSN cultures (Figure 1F). Based on these results, and those from Northern, PAGE, and Western analyses, we concluded that the versican antisense significantly reduced versican production by FRSMC in vitro.

Versican Antisense Induced Changes in Cell Phenotype
LVaSN cells at both low and high density were more flattened and spread than LXSN cells (Figure 2A through 2D). LVaSN cells proliferated at a rate half that of LXSN cells (Figure 2E), and, in a scrape wound assay, showed a significantly slower rate of migration during the first 12 hours compared with LXSN cells (Figure 2F). At later time periods, the migration rate was similar but the initial difference was maintained. The flattened LVaSN cells were also more adhesive, as measured by a trypsin resistance assay,21 and had reduced cell coats, as measured by a particle exclusion assay21 (data not shown). These morphological and physiological features were similar to those found previously for LV3SN.21

Versican Antisense Increased Tropoelastin mRNA and Elastic Fiber Deposition In Vitro
Tropoelastin mRNA levels of LVaSN cells were significantly increased compared with LXSN cells (Figure 3A), and 7 day LVaSN cultures stained more intensely for elastin than LXSN cultures (Figure 3B). Morphometric analysis confirmed significantly (P<0.001) increased immunostaining of elastin, and the reciprocal finding of decreased immunostaining of CS (Figure 3C). Metabolic labeling with [3H]-valine showed that insoluble elastin in 7-day LVaSN cultures was significantly increased (Figure 4A), and immunostaining of 3-week cultures of LVaSN cells showed increased deposits of elastin and elastic fibers (Figure 4B, top). CS add-back decreased both insoluble and immunostained elastin (Figure 4A and...
The increase in extracellular elastin in LVaSN cultures was confirmed by TEM of 3-week cultures. Elastin volume fraction, measured by point counting of extracellular elastin in the cell layer, was 10-fold higher in LVaSN (4.3%) compared with LXSN (0.4%) ($P<0.005$).

Versican Antisense Increased S-Gal/EBP
LVaSN cells cultured for 48 hours stained more intensely with anti–S-Gal (Figure 5A) and with anti-EBP (Figure 5B, upper panels) than did LXSN cells. The mean percentage increase for anti-EBP staining intensity, measured by point counting of extracellular elastin in the cell layer, was 10-fold higher in LVaSN (4.3%) compared with LXSN (0.4%) ($P<0.005$).

Western blot analysis of cell lysates with anti–P-Gal showed that LXSN and LVaSN had similar amounts of the 88-kDa β-Gal precursor and the mature 64-kDa form of the active enzyme. In contrast, the 67-kDa catalytically inactive spliced variant of β-Gal (S-Gal), detected by anti–P-Gal, anti–S-Gal, and by anti-EBP, was increased in LVaSN cell lysates compared with LXSN (Figure 5D). Add-back of CS decreased the 67-kDa S-Gal/EBP in both LVaSN and LXSN cells but had no effect on precursor or active β-Gal (Figure 5D).

Versican Antisense–Expressing Cells Formed a Versican-Depleted and Elastic Fiber–Rich Neointima
Seven-day neointima formed from LXSN cells was characterized by stellate or rounded FRSMC embedded in a matrix that was high in versican (Figure 6A), relatively depleted of EBP (Figure 6C), and with few elastin deposits (Figure 6E). Seven-day neointima formed from LVaSN cells was charac-

![Figure 4](image-url)  
*Figure 4.* A, Insoluble elastin, determined by quantitative analysis of [3H]-valine–labeled NaOH-insoluble residues. LVaSN had significantly (**$P<0.001$**) more insoluble elastin than LXSN cultures. Add-back of CS (400 μg/mL) significantly reduced (**$P<0.001$**) insoluble elastin in both LVaSN and LXSN. B, Three-week LXSN and LVaSN cultures immunostained for elastin and visualized with Texas Red. LXSN cultures contained scattered deposits of elastin, LVaSN cultures contained more numerous deposits and nascent fibers. Daily addition of CS (200 μg/mL) to LXSN and LVaSN cultures for 3 weeks diminished elastin staining and reduced fiber deposition in LVaSN cultures. Magnification, ×600.

![Figure 5](image-url)  
*Figure 5.* A, Low-density LXSN and LVaSN cells cultured for 48 hours and immunostained with anti–S-Gal and visualized with FITC (green). Magnification, ×400. B, Low-density LXSN and LVaSN cells, cultured in the absence and presence of versican (200 μg/mL), and immunostained with anti-EBP and visualized with Texas Red. Magnification, ×600. C, Mean ODs (±SD) of individual LXSN and LVaSN cells (clones 1 and 2) treated with versican (+V) and immunostained with anti-EBP. Versican add-back significantly reduced (*$P<0.001$*) EBP staining in the LVaSN clones (n=15). D, Western blotting with anti–P-Gal antibody detecting β-Gal–related species (88-kDa precursor, 67-kDa inactive S-Gal, and 64-kDa mature enzyme), and with anti–S-Gal and anti-EBP antibodies detecting 67-kDa S-Gal/EBP in lysates of LXSN and LVaSN cultured in the absence and presence of exogenous CS (400 μg/mL).
terized by reduced versican staining (Figure 6B), increased EBP staining (Figure 6D), and numerous small deposits of elastin preferentially distributed at cell surfaces (Figure 6F). Morphometric analysis showed a nonsignificant trend toward reduced thickness of neointima formed from the LVaSN cells (data not shown). Cell proliferation indices, determined from BrdUrd labeling, were not significantly different in the 2 groups (data not shown).

Sections of 28-day LXSN neointima, stained with Toluidine Blue or orcein, showed punctate elastin deposits in a myxoid matrix surrounding stellate cells (Figure 7A, 7C, and 7E). Twenty-eight-day neointima formed by LVaSN cells was more compact with significantly more elastin (Figure 7B, 7D, and 7F), which, in regions of highest content, was organized into wavy circumferential fibers and lamellae between elongated cells (Figure 7B and 7D), resembling a developing media. Similar to 7-day neointima, there was a nonsignificant trend toward decreased intimal thickness of neointima formed from LVaSN cells (data not shown).

These structural and morphological differences were confirmed by analysis of TEM micrographs of 28-day neointima (Figure 8A and 8B). Point-counting of cells and extracellular matrix components (Figure 8C) showed that neointima formed by LVaSN cells had a significantly ($P<0.001$) higher elastin volume fraction (36.5%), and a significantly ($P<0.01$) lower matrix space volume fraction (12.1%), compared neointima formed from LXSN cells (19.4% and 18.2%, respectively). Cell and collagen volume fractions were not significantly different.

Discussion

The results of this study demonstrate the central importance of versican in controlling vascular cell phenotype and composition of the extracellular matrix, in vitro and in vivo. Retroviral transduction of cultured FRSMC with a versican antisense sequence decreased production and deposition of versican, induced a flattened morphology, slowed proliferation and migration, increased tropoelastin and its chaperone S-Gal/EBP, and increased elastin deposition and fiber formation. Seeding of versican antisense expressing cells into balloon injured rat carotid arteries resulted in neointima with a highly ordered structure, reduced versican content, and increased S-Gal/EBP and elastic fiber content.

A number of studies have reported an inverse relationship between versican and elastogenesis. In versican-rich tissues, such as in restenotic lesions and the ductus arteriosus, elastic fibers are scarce. Conversely, neonatal SMC that produce little CS, show a high level of tropoelastin synthesis.

Figure 6. Light micrographs of 7-day neointimae formed from LXSN (top) and LVaSN (bottom) cells seeded into balloon-catheter injured rat carotid arteries and immunostained for versican (A and B) and EBP (C and D) and stained with Toluidine Blue to show elastin (E and F). Neointima formed by LVaSN cells contained less versican, increased EBP, and increased elastin deposits at cell surfaces (dark blue deposits indicated by arrows) compared with LXSN neointima. Magnifications: $\times$1200 (A and B); $\times$750 (C and D); $\times$2500 (E and F).

Figure 7. Light micrographs of 28-day neointimae formed from LXSN (A, C, E) and LVaSN (B, D, F) cells seeded into balloon-catheter injured rat carotid arteries and stained with Toluidine Blue (A through D) and orcein (E and F). LXSN neointima contained rounded or stellate FRSMC embedded in an abundance of proteoglycans (purple staining indicating metachromasia) and scattered elastin deposits (dark blue). Neointima formed by LVaSN cells contained elongated and mostly circumferentially arranged FRSMC in a compact matrix with reduced proteoglycan content, and increased elastin organized into wavy fibers and lamellae. Magnifications: $\times$700 (A and B); $\times$2500 (C through F).
Impaired elastogenesis also occurs in Hurler disease and Costello syndrome, in which galactosugar-containing GAGs accumulate around cells in the skin and other organs, including vessels. Accumulation of galactosugars has been demonstrated to negatively interfere with the function of S-Gal/EBP and exposure of fibroblasts to high levels of CS results in shedding of S-Gal/EBP from cell surfaces and disruption of elastic fiber formation. Chondroitin ABC lyase digestion has been used to reduce excess CS and successfully correct elastin and EBP deficiencies in skin fibroblasts from Costello patients.

Our finding of similar amounts of the 88-kDa β-Gal precursor and the 64-kDa form of the active enzyme in LXSN and LVaSN cells indicates that the change in S-Gal/EBP in VSMC is not at the level of gene expression. Rather, our data, demonstrating increased elastin and fiber formation in VSMC cultures and neointima depleted in versican and decreased elastin deposition and S-Gal/EBP content following exposure to exogenous CS or versican, are consistent with the model of versican-mediated inhibition of elastic fiber assembly operating through galactosugar modulation of S-Gal/EBP levels.

Other recent studies have explored the relationship between versican and elastogenesis. Most notably, overexpression of V3 (lacking CS chains) by FRSMC results in upregulation of tropoelastin transcription and increased elastic fiber assembly, both in vitro and in vivo, as well as phenotypic changes similar to those found for the versican-depleted antisense-expressing cells used in this study. Overexpression of V3 has also been shown to reverse the impaired elastogenesis in fibroblasts from Costello syndrome and Hurler disease patients. Although the basis for this V3-induced elastogenesis is not clear, it has been hypothesized that V3, which retains the hyaluronan-binding domain common to all versican variants, competitively removes the CS-bearing versican variants from HA in the pericellular coat and allows for increased residence time for cell surface EBP and elastic fiber assembly. V3 may also interact with elastic fiber components, such as fibrillin-1 or the fibulins, to promote elastogenesis, but such interaction does not explain the findings of this present study where all versican variants were significantly reduced by the antisense, including any endogenous V3. Our findings support the first hypothesis, namely that it is the depletion of versican that is permissive for elastic fiber assembly. We cannot discount, however, that versican influences elastin stability and turnover through modulation of protease activity.

The increased deposition of elastin in cultures of versican-depleted cells was accompanied by increased tropoelastin mRNA. A high level of tropoelastin expression also occurs in neonatal SMC that produce little or no versican. The mechanism responsible for this upregulation is not known but could be attributable to, either or both, an increase in the transcription rate of the elastin gene or increased stability of tropoelastin message. Current studies from our laboratories, including those suggesting that forces generated during changes in the cell shape may induce signals stimulating elastin gene expression, will likely resolve this question.

The versican-depleted antisense-expressing cells seeded into the balloon-injured arteries formed an elastin-rich and highly structured neointima with a comparatively dense extracellular matrix. The latter is consistent with the reduction in versican and a correspondingly reduced level of hydration of the matrix, whereas the increased elastin content is consistent with other studies discussed above showing that removal of galactosugars promotes elastic fiber assembly. The increase in S-Gal/EBP in the neointima formed from antisense-expressing cells is similarly consistent with a reduced content of versican.

Interestingly, and in contrast to the elastic fibers seen in vitro, the neointimal elastic fibers were organized in layers between circumferentially arranged and generally elongated SMC. We postulate that this regular organization may be attributable to mechanical forces associated with systole and diastole. These fibers may in turn have regulatory functions of maintaining a stable and highly structured neointima. In this regard, it is notable that disruption of elastin assembly, by elastin gene knockout, induces subendothelial VSMC proliferation and obstruction of the lumen.

Finally, the experimental model used in this study to form a versican-depleted neointima may be especially valuable for other studies, including testing the role of versican in binding atherogenic lipids and other factors associated with the development of atherosclerosis. Modulation of versican levels by antisense or other approaches, including V3, may also offer novel therapeutic approaches for remodeling other elastin-deficient tissues.
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References

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