

# Proteoglycans in Atherosclerosis and Restenosis

## Key Roles for Versican

Thomas N. Wight, Mervyn J. Merrilees

**Abstract**—The proteoglycan versican is one of several extracellular matrix (ECM) molecules that accumulate in lesions of atherosclerosis and restenosis. Its unique structural features create a highly interactive molecule that binds growth factors, enzymes, lipoproteins, and a variety of other ECM components to influence fundamental events involved in vascular disease. Versican is one of the principal genes that is upregulated after vascular injury and is a prominent component in stented and nonstented restenotic lesions. The synthesis of versican is highly regulated by specific growth factors and cytokines and the principal source of versican is the smooth muscle cell. Versican interacts with hyaluronan, a long chain glycosaminoglycan, to create expanded viscoelastic pericellular matrices that are required for arterial smooth muscle cell (ASMC) proliferation and migration. Versican is also prominent in advanced lesions of atherosclerosis, at the borders of lipid-filled necrotic cores as well as at the plaque-thrombus interface, suggesting roles in lipid accumulation, inflammation, and thrombosis. Versican influences the assembly of ECM and controls elastic fiber fibrillogenesis, which is of fundamental importance in ECM remodeling during vascular disease. Collectively, these studies highlight the critical importance of this specific ECM component in atherosclerosis and restenosis. (*Circ Res.* 2004;94:1158-1167.)

**Key Words:** proteoglycans ■ versican ■ extracellular matrix ■ atherosclerosis ■ smooth muscle cells

Versican is a chondroitin sulfate proteoglycan (CSPG) that is present in the extracellular matrix (ECM) of normal blood vessels and increases dramatically in all forms of vascular disease.<sup>1,2</sup> A number of reports within the last few years have documented a significant involvement of versican in lesions of atherosclerosis and restenosis and these observations, coupled to those that demonstrate that this ECM proteoglycan regulates many of the events that contribute to the formation of atherosclerotic and restenotic lesions, highlights the critical importance of versican in the pathogenesis of vascular disease.

Versican is one of many proteoglycans identified in vascular tissue or synthesized by vascular cells<sup>3</sup> and together with biglycan, decorin, and perlecan constitute the bulk of the proteoglycans found in the interstitial space.<sup>2</sup> Versican interacts with hyaluronan, a long chain high molecular weight glycosaminoglycan (GAG), that is also present in the extracellular matrix (ECM) of blood vessels and increases as versican in vascular disease.<sup>4</sup>

The versican gene and protein follow a domain template. The amino-terminal globular domain (G1) binds hyaluronan, and the carboxy-terminal globular domain (G3) resembles the selectin family of proteins, consisting of a C-type lectin adjacent to two epidermal growth factor (EGF) domains and a complement regulatory region. The

middle region of the versican core protein is encoded by two large exons that specify the chondroitin sulfate attachment regions of versican (Figure 1). These highly interactive domains are the basis for the name given to this “versatile” molecule.<sup>5</sup>

Versican exists in at least four different isoforms created by alternative splicing of mRNA from a single gene. RNA splicing occurs in the two large exons, 7 and 8, that encode the GAG attachment sites, giving rise to V0, V1, V2, and V3 forms of versican.<sup>6</sup> These variants differ in the length of the core proteins and number of attached GAGs (Figure 1).<sup>7</sup> Expression of the versican gene is regulated by a promoter that harbors a typical TATA box and potential binding sites for several transcription factors such as AP2, CCAAT enhancer protein, and cAMP-responsive elements.<sup>8</sup> Regulation of versican core protein synthesis can be at transcriptional<sup>8</sup> or posttranscriptional levels (J. Lemire, C. Tsoi, S. Bressler, and T. Wight, unpublished observations, 2004).

Typically, the chondroitin sulfate (CS) chains attached to the core protein differ in size and composition depending on the tissue source or culture conditions. They vary in size from 25 to 80 kDa and contain different ratios of chondroitin 6-sulfate to chondroitin 4-sulfate.<sup>9</sup> Their length and composition are regulated and controlled by different stimuli (see following sections).

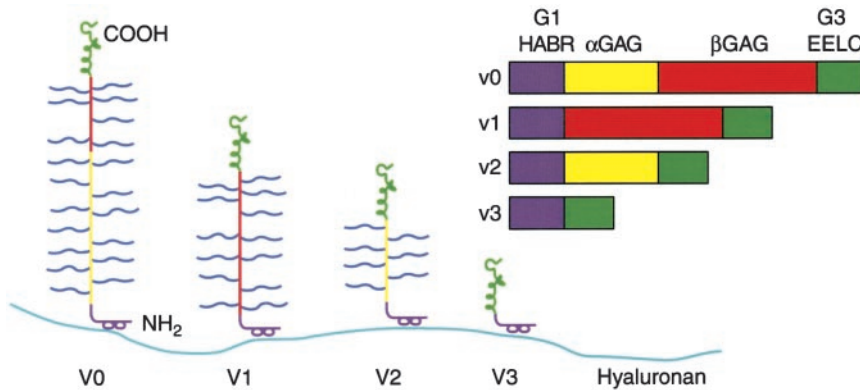
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**Figure 1.** Model of the different isoforms generated by alternative splicing of the mRNA transcript for versican. All isoforms possess a hyaluronan binding region and thus are capable of forming different-sized versican-hyaluronan aggregates, which in turn determine, in part, tissue volume. Different colors denote specific domains in the gene and in the protein product. Purple indicates G1 hyaluronan binding region (HABR); yellow, the  $\alpha$  GAG (exon 7) and protein product; red,  $\beta$  GAG (exon 8) and the protein product; green, 63 including two epidermal growth factor repeats (EE), a lectin binding domain (L), and a complement regulatory region (C). Glycosaminoglycan chains are shown in blue. Reproduced from Wight TN. Versican: a versatile extracellular matrix proteoglycan in cell biology. *Curr Opin Cell Biol.* 2002;14:617–623, with permission.

glycan chains are shown in blue. Reproduced from Wight TN. Versican: a versatile extracellular matrix proteoglycan in cell biology. *Curr Opin Cell Biol.* 2002;14:617–623, with permission.

### Versican in Normal Blood Vessels

Versican is the principal CSPG in blood vessels<sup>10</sup> and immunohistochemistry reveals that versican is prominent in the intima and adventitia of most arteries and veins.<sup>11</sup> It is likely that accumulation of versican in the normal arterial intima is mainly responsible for the proteoglycan-rich nature of this layer.<sup>12</sup> Versican interacts with hyaluronan and link protein to form high molecular weight stable aggregates<sup>13–15</sup> that fill the ECM space not occupied by the other fibrous proteins such as collagens and elastic fibers. These complexes create a reversibly compressive compartment and provide a swelling pressure within the ECM that is offset by collagen and elastic fibers.<sup>16,17</sup> Early biochemical studies of bovine<sup>18,19</sup> and human aorta<sup>20</sup> identified a large CSPG that resembled the principal CSPG synthesized by cultured arterial smooth muscle cells (ASMCs)<sup>21,22</sup> and later identified as versican.<sup>1,9</sup> Recent studies identified specific cleavage products of versican in human aorta that contain the G1 domain of versican. Further studies identified the enzymes that cleaved aortic versican as members of the ADAMS-TS family.<sup>23,24</sup> Other proteases in arteries that are known to degrade versican include matrilysin<sup>25</sup> and plasmin.<sup>26</sup> These findings are consistent with the presence of multiple sizes of versican and versican fragments within vascular tissue.

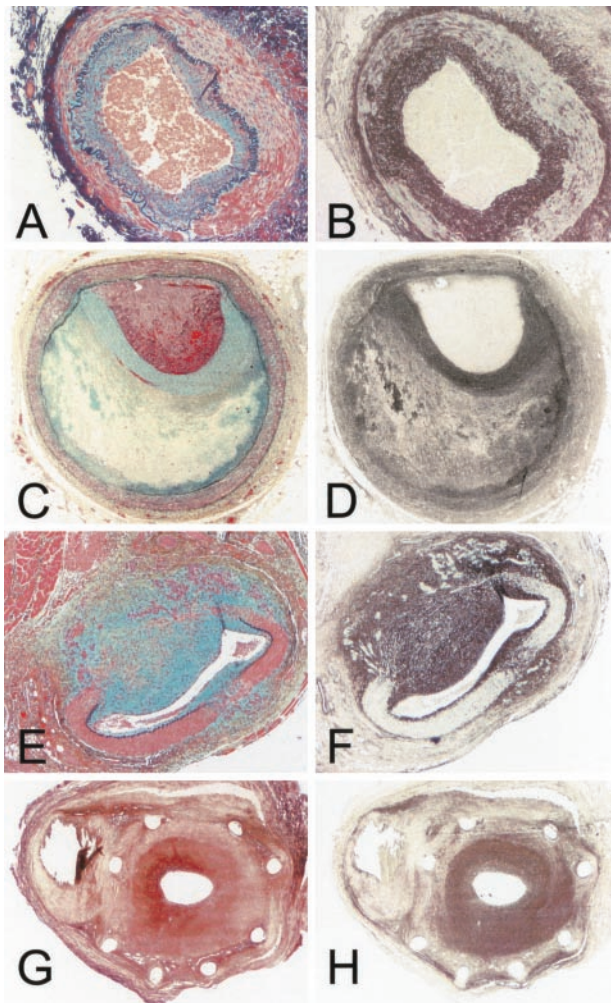
### Versican in Atherosclerosis and Restenosis

#### Accumulation and Role in Lipid Retention

The bulk of information regarding involvement of versican in naturally occurring atherosclerosis in human and experimental animals comes from immunohistochemical studies.<sup>25,27–33</sup> Versican is present in early intimal thickenings that characterize developing atherosclerotic lesions and are primarily associated with ASMCs (Figures 2A and 2B). In a gene array study of neointimal formation in graft healing, versican was one of only 13 genes selectively upregulated<sup>34</sup> and found to accumulate in early graft lesions (R. Kenagy, J. Fischer, A. Clowes, and T. Wight, unpublished observations, 2004). Additionally, versican tends to accumulate in human vessels susceptible to atherosclerosis such as the coronary artery and saphenous veins used for grafting when compared with those resistant such as internal mammary and radial arteries.<sup>11</sup> Whereas versican appears to contribute to intimal expansion

and lesion progression, versican degradation may be part of lesion regression. In a high blood flow model of intimal atrophy in polytetrafluoroethylene (PTFE) aortoiliac grafts, extracts of 4-day high-flow intimas degraded more vascular versican than similar extracts from 4-day low-flow intimas.<sup>26</sup> This activity was shown to be due to elevated plasmin levels.

Versican is present throughout advanced lesions of atherosclerosis as well. A large CSPG has been isolated from atherosclerotic lesions of large hydrodynamic size with a core protein ranging in size from 160 to 245 and containing a mixture of chondroitin 6-sulfate and chondroitin 4-sulfate chains with chondroitin 6-sulfate predominating.<sup>20,35–38</sup> Versican is prominent at the edges of the necrotic core in more advanced lesions of atherosclerosis and in close proximity to deposited lipoproteins,<sup>25,29,30</sup> suggesting a role in the retention of lipoproteins in the vessel wall. The response to retention hypothesis of atherosclerosis,<sup>39</sup> based on pioneering work performed in the 1970s and 1980s<sup>40–44</sup> invokes a critical role in atherogenesis for the retention of lipoproteins by ECM proteoglycans. A number of studies have shown that versican as well as other proteoglycans such as biglycan are associated with lipoprotein deposits in human atherosclerotic lesions<sup>25,27,29,31</sup> and in lipid induced lesions in experimental animals.<sup>30,45,46</sup> Large chondroitin sulfate–lipoprotein complexes have been isolated from human atherosclerotic lesions<sup>47,48</sup> and arterial CSPGs interact with lipoproteins.<sup>38,43,49,50</sup> Analysis of binding constants of CSPGs from lesions reveals that multiple LDL particles can bind to a single CS chain.<sup>48,49,51</sup> Thus, GAG chain length is a determining factor in lipid binding. Vascular injury produces elongated GAG chains on the large vascular CSPG promoting LDL binding.<sup>51</sup> Conditions that promote CS chain elongation in ASMCs such as cell proliferation,<sup>52</sup> treatment of the cells with oxidized LDL<sup>53</sup> and transforming growth factor- $\beta$  (TGF- $\beta$ )<sup>54</sup> also cause increased binding of versican to LDL. In addition, exposure of human ASMCs to nonesterified free fatty acids, as occurs in diabetes, increases the production of proteoglycans including versican, which then binds LDL more effectively.<sup>55</sup> In vitro studies of versican isolated from cultured ASMCs demonstrate that this proteoglycan binds to LDL with saturable kinetics and with a binding affinity for versican of  $2.3 \times 10^{-8}$  mol/L.<sup>56,57</sup> The composition of the CS chains of versican may effect LDL interaction as well. CS



**Figure 2.** A, Section from a human artery with intimal thickening stained with a modified Movats stain that shows proteoglycans as blue, collagens as yellow, and elastic fibers as dark purple;  $\times 20$ . Note enrichment for proteoglycans in the thickened intima. B, Adjacent section to that shown in A, immunostained for versican. Note that the thickened intima is enriched in versican;  $\times 20$ . C, Section from an eroded human coronary atherosclerotic plaque stained with a modified Movats stain showing a proteoglycan rich layer adjacent to an occluding thrombus;  $\times 20$ . Reproduced from Kolodgie FD, Burke AP, Farb A, Weber DK, Kutys R, Wight TN, Virmani R. Differential accumulation of proteoglycans and hyaluronan in culprit lesions: insights into plaque erosion. *Arterioscler Thromb Vasc Biol.* 2002;22:1642–1648, by permission of the American Heart Association ©2002. D, Adjacent section to that shown in C immunostained for versican. Note the proteoglycan-enriched layer adjacent to the thrombus is enriched in versican;  $\times 200$ . E, Section from a human temporal artery pseudoaneurysm stained with a modified Movats stain showing proteoglycans in blue, collagens in yellow, and elastic fibers in dark purple. Note interruption of the elastic fibers and accumulation of proteoglycans in the developing lesion;  $\times 20$ . Sections kindly provided by Drs Alan Burke and Renu Virmani of the Armed Forces Institute of Pathology, Washington, DC. F, Adjacent section to that shown in E immunostained for versican. Note the significant accumulation of versican in those regions where the lesions are developing;  $\times 20$ . Section kindly provided by Drs Alan Burke and Renu Virmani, Armed Forces Institute of Pathology, Washington, DC. G, Section from an in-stent human restenotic coronary artery stained for hyaluronan (red) using a biotinylated hyaluronan binding probe. Note the stent wires separate the old and new lesion and significant accumulation of hyaluronan in the new lesion;  $\times 20$ . Section kindly provided by Drs Andrew Farb and Renu Virmani of the Armed Forces Insti-

enriched in the 6-O sulfate isomer binds LDL more avidly than those enriched in the 4-O sulfate isomer.<sup>58</sup> Furthermore, the charge density on the GAG influences LDL interactions in that the degree of sulfation affects lipoprotein interaction with proteoglycans.<sup>59</sup>

Although a number of studies indicate that versican is clearly capable of binding to LDL, versican is generally not detected in the lipid-rich center of the necrotic core nor does it colocalize with apoE or apoB epitopes in both human and mouse atherosclerotic lesions. Instead other proteoglycans such as biglycan, a DSPG, and perlecan, a HSPG, together with macrophages, tend to predominate.<sup>31,60</sup> For example, in the murine apoE-null atherosclerotic lesion, biglycan and perlecan were found to best colocalize with lipoproteins,<sup>60</sup> raising questions about the importance of versican in lipid retention in this animal model. Species differences do exist regarding the types and amounts of proteoglycans in the vasculature. The murine vasculature is enriched in heparan sulfate proteoglycans, whereas the human vasculature contains a large proportion of CS and DS proteoglycans.<sup>61,62</sup> The reasons for these differences are not clear but may indicate fundamental species differences in the nature of the ECM in atherosclerotic lesions between mice and humans. What is clear is that multiple types of proteoglycans can interact with lipoproteins, and what is important is that it may not necessarily be the type of proteoglycan present but where and how much of a particular proteoglycan is found during the lipid phase of this disease.

Although the precise structure(s) in the proteoglycans responsible for LDL binding has yet to be determined, versican and biglycan binding sites in human apoprotein B of LDL have been located at residue 3363,<sup>56</sup> whereas different binding sites exist for apoB 48.<sup>63</sup> Furthermore, transgenic mice overexpressing a defective proteoglycan-binding apoprotein when fed a high-fat diet failed to develop atherosclerosis after 20 weeks.<sup>64</sup> These results indicate that subendothelial retention of apoprotein B-100-containing lipoproteins by proteoglycans is a critical step in the early stages of atherosclerosis. The precise role of versican in these early phases remains to be determined. Furthermore, lipoproteins bound to proteoglycans are more sensitive to modifications such as oxidation and enzymatic hydrolysis, thereby affecting their potential atherogenicity.<sup>65–67</sup> It should also be pointed out that lipid content of the lipoprotein also affects their interaction with proteoglycans,<sup>66,67</sup> indicating the probability of multiple interactive sites between these two sets of molecule.

In addition to regulating the extracellular retention of lipoproteins, versican may play a role in intracellular lipid accumulation as well. Chondroitin sulfate proteoglycan-LDL

tute of Pathology, Washington, DC. H, Adjacent section as shown in G immunostained for versican. Note excessive accumulation of versican in the new lesion on the luminal side of the stent wires. Immunostaining for versican parallels to a large extent the staining for hyaluronan indicating the likelihood of large hyaluronan-versican complexes filling the ECM of these lesions;  $\times 20$ . Section kindly provided by Drs Andrew Farb and Renu Virmani of the Armed Forces Institute of Pathology Washington, DC.



complexes are taken up rapidly by macrophages<sup>65,68</sup> and ASMCs<sup>69</sup> and recent studies show that ASMCs internalize versican-LDL complexes through both LDL receptor and LDL receptor-related protein pathways.<sup>70</sup> This uptake leads to accumulation of lipid and formation of foam cells which characterize lipid-filled atherosclerotic plaques. Thus, versican may play fundamental roles in both the extracellular and intracellular retention of lipoproteins in atherogenesis.

### Role in Inflammation

Accumulation of a versican-hyaluronan-enriched ECM may also influence the retention of inflammatory cells that also form part of the developing atherosclerotic lesion.<sup>71</sup> For example, hyaluronan can serve as an attachment ligand for macrophages<sup>72</sup> and lymphocytes through CD44-mediated interactions.<sup>4</sup> Versican, which binds to hyaluronan, can also bind to CD44,<sup>73</sup> suggesting that both versican and hyaluronan may stabilize CD44-dependent interactions of inflammatory cells. *In vitro* studies show that both versican and hyaluronan support macrophage adhesion.<sup>72,74,75</sup> Additionally, versican interacts with inflammatory chemokines and partly regulates their activity.<sup>73,76–78</sup> It is of interest that one of the spliced variants of CD 44 that contains CS interacts with interferon  $\gamma$ ,<sup>79</sup> suggesting a potential role for molecules like versican to retain inflammatory cytokines at the ASMC surface.

### Thrombosis and the Eroded Plaque

Versican and hyaluronan are also present at the plaque thrombus interface in advanced human atherosclerotic lesions<sup>32</sup> (Figures 2C and 2D), suggesting a possible role in thrombosis. Recent experiments have shown that versican promotes platelet adhesion at low shear rates and cooperates with collagens to promote platelet aggregation.<sup>80</sup> The presence of hyaluronan with versican at these sites also supports a prothrombotic role for hyaluronan because platelets can stick to hyaluronan through CD44 receptors.<sup>81</sup> Such findings indicate that versican-hyaluronan complexes may serve as ancillary platelet ligands to the other known ligands to influence platelet deposition after rupture of the atherosclerotic plaques. In addition, versican at the plaque thrombus interface may in part regulate the water content of this region of the lesion and in turn promote coagulation because water transfer is linked to anticoagulation activity of molecules such as the CS in versican.<sup>82</sup> Versican isolated from advanced type IV atherosclerotic lesions prone to thrombosis is under-sulfated relative to versican from nondiseased aortas. Decreased sulfation of versican may predispose the lesions to thrombosis by disrupting osmotic regulation and limiting antithrombin activity.

### Aneurysms

Aortic aneurysms are a frequent complication of severe atherosclerosis and constitute an important clinical problem.<sup>83</sup> Versican V0 mRNA is decreased by 40% in human abdominal aortic aneurysms with significantly reduced immunostaining for versican.<sup>10</sup> Some of this loss could be due to selective versican degradation as versican degradative enzymes such as plasmin increase in association with aneurysmal expansion.<sup>84,85</sup> Thus, late phase aneurysmal development

may involve selective loss of versican. Pseudoaneurysms of the temporal artery show increased immunostaining for versican in areas where there is elastic fiber interruption,<sup>86</sup> indicating a possible early involvement of versican in remodeling involving elastogenesis (Figures 2E and 2F). Interestingly, ASMCs cultured from aneurysmal aortas synthesize elevated levels of versican.<sup>87</sup> In addition, a recent gene mapping study of familial thoracic aortic aneurysms and dissections identified a gene locus for this disease at 5q13-14,<sup>88</sup> which is the chromosomal segment that contains the versican gene.<sup>89</sup> However, sequencing of versican DNA from affected individuals failed to reveal any variation that could account for the phenotype. It remains to be seen if changes in any of the versican variants could account for susceptibility to aneurysms. Although versican changes in aneurysms are well documented, it is still not clear whether these changes are causative or secondary to other ECM abnormalities. However, a connection between versican and elastic fiber assembly as discussed below supports a role for versican in maintenance of elastic fiber assembly and blood vessel wall stability.

### Increase in Response to Vascular Injury and the Restenotic Lesion

Accumulation of versican typifies early lesion development in response to vascular injury. Versican increases in the intima in the early phases of response to balloon injury and in vein graft repair and contributes to intimal expansion after vascular trauma.<sup>26,45,46,90–94</sup> Interference with versican expression either by antibodies to TGF- $\beta$ <sup>90</sup> or antisense<sup>92</sup> blocks intimal expansion after injury.

Versican is markedly increased in both stented and non-stented restenotic lesions in humans<sup>95–97</sup> (Figures 2G and 2H). Accumulation occurs primarily in the ASMC hypercellular myxoid regions of the lesions that have a very low content of collagen and elastic fibers. Hyaluronan which associates with versican also accumulates in the versican-rich regions of these lesions<sup>4,98</sup> and forms high molecular weight hydrophilic complexes that trap water and cause tissue swelling. The rapid expansion of restenotic lesions could be due, in part, to the swelling pressure brought on by accumulation of versican-hyaluronan complexes. Conversely, loss or breakdown of the hyaluronan-versican complex could lead to expulsion of water and tissue shrinkage with reduction in arterial circumference. Interestingly, collagen gels impregnated with hyaluronan show CD-44-dependent contraction when populated by ASMCs.<sup>99</sup> The involvement of versican in regulating the ASMC's ability to contract their matrices has not been investigated.

### Regulated Synthesis of Versican and Regulation of Vascular Cell Phenotype

Arterial smooth muscle cells are the principal source of versican in both arteries and veins,<sup>9,21,22,34,92,100–106</sup> although endothelial cells<sup>107–110</sup> and myofibroblasts derived from adventitial fibroblasts<sup>45</sup> also have been shown to synthesize versican. ASMCs contain mRNA transcripts for V0, V1, and V3.<sup>103</sup> The synthesis of versican by ASMCs is regulated by specific factors that contribute to atherogenesis. PDGF, a major mitogen for these cells, upregulates versican core

protein synthesis without affecting core protein synthesis of biglycan and decorin.<sup>9,101,105,111</sup> This upregulation depends on endogenous tyrosine kinase activity because genistein completely blocks PDGF-induced synthesis of versican.<sup>101</sup> This growth factor also affects the posttranslational processing of versican by stimulating CS chain elongation.<sup>9</sup> This effect, however, is not restricted to versican because PDGF also causes GAG chain elongation of decorin and biglycan.<sup>111</sup> Such observations suggest that pathways that control versican core protein synthesis and CS and DS synthesis are regulated differently. For example, inhibiting tyrosine kinase activity does not affect CS chain elongation by PDGF in ASMCs.<sup>101</sup> An involvement of MAPKK in the regulation of versican synthesis by ASMCs has been confirmed in that angiotensin II stimulation of versican synthesis requires MAPKK.<sup>112</sup> Other cytokines known to regulate versican synthesis include TGF- $\beta$ ,<sup>9</sup> EGF,<sup>113</sup> and bFGF (K. Braun, S. Potter-Perigo, and T. Wight, unpublished observations, 2004). Interestingly, leukotriene, LTD4 and EGF treatment of ASMCs increases V0 versican synthesis over V1 versican,<sup>113</sup> indicating differential regulation of versican isoforms by specific cytokines. The larger V0 form would occupy larger ECM volumes and contribute to greater ECM expansion.

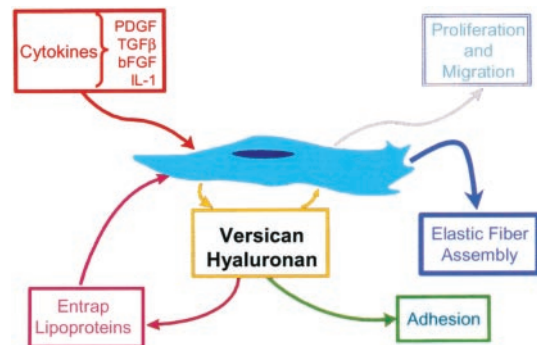
Versican synthesis by ASMCs is also regulated by mechanical forces such as cell stretching. For example, low amplitude biaxial strain induces a time-dependent increase in the synthesis of versican,<sup>106</sup> while at the same time causing decreases in the expression of decorin. Elevated versican synthesis is accompanied by increases in versican-hyaluronan aggregation, indicating a direct influence of mechanical stretch on ECM remodeling. It may be that such changes create a more malleable, deformable, hygroscopic ECM surrounding cells to accommodate the stretch on the cell so as to prevent cell damage.

Although a number of chemical and mechanical factors stimulate ASMC versican expression, a few inhibit. Interleukin (IL)-1 $\beta$  decreases versican expression while increasing the synthesis of decorin.<sup>114,115</sup> Because macrophage-derived IL-1 is capable of causing these changes, it is likely that macrophages can also regulate versican content of developing lesions. On the other hand, versican is generally absent in areas where macrophages accumulate in atherosclerotic lesions,<sup>25,27,30,31</sup> suggesting macrophages have a role in versican degradation.

Although ASMCs are a major source of versican in vascular lesions, endothelial cells also synthesize versican in a regulated fashion. TGF- $\beta$  increases versican expression in bovine aortic endothelial cells<sup>109</sup> and versican synthesis is down-regulated when endothelial cells are stimulated to migrate.<sup>108</sup> More recent studies suggest that a specific isoform of versican, V3, is increased by proangiogenic cytokines such as VEGF and TNF- $\alpha$  and associated with a neoangiogenic endothelial phenotype.<sup>110</sup>

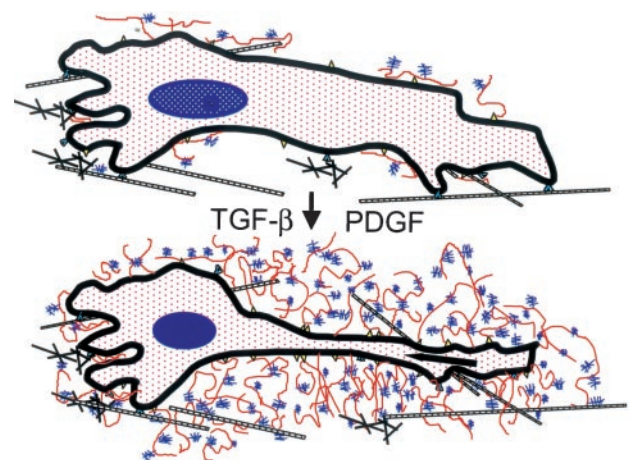
### Effects on Vascular Cell Adhesion, Proliferation, and Migration

The finding that mitogens such as PDGF lead to increased synthesis of versican suggests that versican may play a role in regulating ASMC proliferation<sup>7</sup> (Figure 3). For example,



**Figure 3.** Summary of the many effects that versican can have on the phenotype of ASMCs. Synthesis of versican is regulated by inflammatory cytokines. Accumulation of versican, together with molecules that associate with versican, influence ASMC adhesion, proliferation, and migration as well as the adhesion, proliferation, and migration of other cells that gain access to the vascular wall. In addition, versican regulates ECM assembly and the entrapment of lipoproteins and thus can be placed at the center of the events that form the basis for the development of the atherosclerotic and restenotic lesions.

molecules that associate with versican such as hyaluronan,<sup>105</sup> CD44,<sup>116</sup> and TSG-6<sup>117</sup> are also upregulated during arterial ASMC proliferation. Versican interacts with hyaluronan and accumulates in the pericellular matrix when ASMCs are stimulated to proliferate and migrate<sup>104</sup> (Figure 4). These changes create a viscoelastic coat around the cells that expands as ASMCs are stimulated to proliferate and migrate (Figure 4). Thus, versican-hyaluronan-rich ECM around the ASMCs allows the cells to change shape to prepare them for proliferation and migration (Figure 4). Blocking the forma-



**Figure 4.** Diagram depicting the formation of a hyaluronan-versican pericellular ECM in response to various stimuli. Diagram is based on experimental studies that show that these pericellular coats form in response to inflammatory stimuli.<sup>104,105</sup> Both PDGF and TGF- $\beta_1$  are known to stimulate hyaluronan (red) and versican (blue) synthesis. Hyaluronan associates with the surface of the arterial smooth muscle cells via hyaluronan binding proteins such as CD44 (yellow). These increases not only facilitate cell shape change to prepare the cells for proliferation and migration, they also are hypothesized to increase ECM volume and mass, interrupt elastic fiber assembly, and provide substrates for platelets and leukocytes. Adapted from Wight TN. Available at: <http://www.glycoforum.gr.jp>.

tion of the versican-hyaluronan pericellular coat, either by blocking antibodies to the hyaluronan receptors such as RHAMM,<sup>118</sup> or using short oligosaccharides of hyaluronan, blocks PDGF induced proliferation of ASMCs.<sup>104</sup> Thus, it would appear that a versican enriched pericellular environment is needed for the proliferative phenotype (Figures 3 and 4).

Another way in which versican could promote vascular cell proliferation is by acting as a mitogen itself through the EGF sequences in the G3 domain. For example, overexpression of G3 minigenes of versican enhances cell proliferation,<sup>119,120</sup> and this effect is due to the EGF sequences in the G3 domain. Such studies point to the potential importance of versican fragments or degradation products controlling cell phenotype. It remains to be seen if this portion of versican is mitogenic for vascular cells.

Because versican is a large hygroscopic molecule, it functions as an antiadhesive molecule<sup>121</sup> through interactions with hyaluronan at the cell surface or with annexin 6.<sup>122</sup> The importance of versican in controlling cell adhesion and cell shape is highlighted by studies where versican isoforms have been overexpressed. For example, overexpression of V3 versican, which lacks CS chains, by retroviral transduction in ASMCs, promotes adhesion, a flattening of the cells, an increase in close contacts, a decrease in cell detachment in response to trypsin, a reduction of pericellular coats, and inhibition of growth and migration.<sup>123</sup> It is hypothesized that the effects of V3 expression on these phenotypic traits are the result of competition for binding sites for V1 versican on hyaluronan associated with the cell surface. Indeed, V3 overexpressing ASMCs have less CS associated with their cell surface than controls (M. Merrilees, A. Hinek, and T. Wight, unpublished observations, 2004). Although it appears that the CS chains are somehow involved in the proliferative phenotype, the mechanism(s) by which versican influences cell proliferation is still not known.

### Role in ECM Assembly

ECM remodeling takes place throughout the different phases of atherosclerosis and restenosis as part of an injury and inflammatory response. These phases involve breakdown and disassembly of various ECM components and reassembly of particular components as part of the pathogenesis of these diseases. The sequence of changes is not unlike what is seen during wound repair in which the early ECM changes are characterized by ECM deposits that create a loose, open, and watery matrix (referred to as a "provisional ECM"<sup>124,125</sup>), which allows for cellular invasion and repair. This provisional matrix is then replaced by a more fibrous ECM enriched in collagens and assorted glycoproteins. What is conspicuously absent in this newly remodeled ECM is elastic fibers. In fact, elastic fibers are conspicuously absent from atherosclerotic and restenotic lesions. The importance of elastic fibers in regulating intimal hyperplasia is highlighted by studies of the elastin knockout mouse. Disruption of elastin synthesis by targeting the promoter and first exon of the tropoelastin gene<sup>126</sup> leads to subendothelial proliferation of ASMCs and obstructive vascular disease. More recently, cells from the elastin knockout animals have been shown to proliferate more rapidly than their normal littermate cells in

vitro but normal proliferation is restored on addition of elastin to the knockout cells.<sup>127,128</sup> Elastin peptides have been shown also to regulate ASMC proliferation and migration.<sup>129</sup> In fact, elastin has been used to dampen the restenotic response in experimental animals.<sup>128</sup> Thus, factors regulating elastic fiber formation may be critical to controlling vascular lesion formation. One factor that appears to inhibit elastic fiber assembly is CS, which is part of the versican molecule.<sup>130</sup> We have found that overexpression of the versican variant that lacks CS in ASMCs leads to changes in tropoelastin expression and accumulation of elastic fibers in long term cultures.<sup>131</sup> Furthermore, when these transduced cells are seeded into balloon injured rat carotid arteries, a compact and highly structured neointima enriched in elastic lamellae develops.

The mechanism by which versican V3 influences tropoelastin expression and formation of elastic fibers is not known but several studies point to an important relationship between regulation of the larger parent CS containing forms of versican and elastin production and assembly. Neonatal ASMCs express little or no versican but express high levels of tropoelastin<sup>102,132</sup> and form elastic fibers *in vitro*.<sup>133</sup> Conversely, elastic fibers are depleted in tissues where versican levels are elevated such as in restenotic arterial lesions<sup>96</sup> or in the intimal cushions of the ductus arteriosus.<sup>130</sup> Such studies suggest that versican inhibits elastic fiber assembly. Although the mechanism(s) for this activity is not known, it is of interest that recent experiments have used V3 transduction of cells taken from patients exhibiting defective elastogenesis to reverse their phenotype and correct the impaired elastogenic phenotype, by influencing an elastin binding protein (EBP) associated with the surface of these cells.<sup>134</sup>

### Is Versican the Culprit?

Versican, with its unique structure and multiple interactions, is one of several proteoglycans that contribute to the pathogenesis of vascular disease. It is well placed to play a central role in the control or modulation of key events in atherogenesis and restenosis. Although further evidence is required to confirm causative roles for versican, there is sufficient evidence to place it at the center of many key processes in vascular pathology. In moderate amounts, and in association with hyaluronan, versican provides a hydrated viscoelastic ECM able to accommodate cyclic mechanical forces imposed on vessels. Elevated levels, however, shift the balance in favor of pathological changes. Increased amounts, especially in the intima, contribute physically to increased intimal thickness and to creation of a pericellular and extracellular matrix that supports cell migration and proliferation and inflammatory cell adhesion. Concomitantly, versican enrichment of the pericellular matrix decreases residence time for cell-associated receptors involved in ECM assembly, such as EBP, thereby decreasing elastic fiber assembly and influencing arterial smooth muscle cell proliferation and the mechanical properties of the expanding intima. Most damaging, however, is alteration in the chondroitin sulfate chains of versican, caused by specific growth factors and cytokines that results in increased retention of lipoproteins and accumulation of lipoproteins. Finally, the presence of versican at the plaque thrombus interface implicates this ECM component in



one of the final phases of atherosclerosis, vessel occlusion. This encompassing hypothesis has yet to be tested but accumulating evidence strongly suggests that versican is a key molecule in atherosclerosis and restenosis and a potential target for therapeutic intervention. Unfortunately, a versican knockout is lethal due to failure of heart and blood vessels to develop normally.<sup>135</sup> Therefore, future directions will be to develop molecular genetic models in which the expression of versican and its variants can be selectively controlled so that the impact of removing this component from the developing atherosclerotic plaque can be assessed directly and the molecular mechanisms responsible for the effects on vascular cell phenotype ascertained.

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