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Arterial remodeling in vascular disease: a key role for hyaluronan and versican

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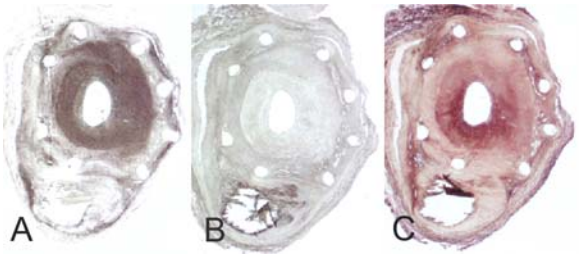
1. ABSTRACT

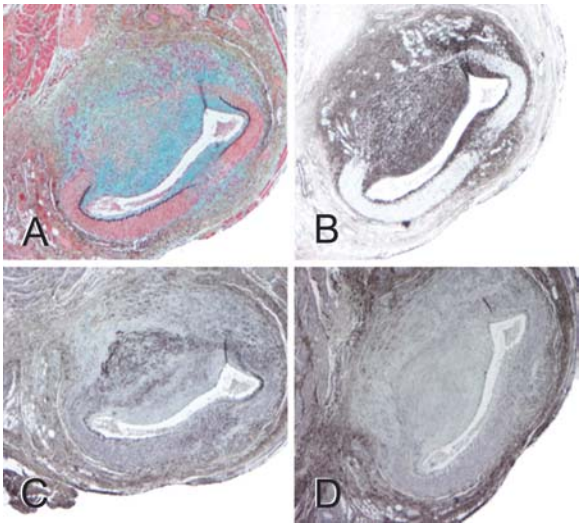
Hyaluronan and versican are extracellular matrix (ECM) macromolecules that are present in low amounts in normal blood vessels, but increase dramatically in vascular disease. These ECM components are particularly enriched in intimal hyperplasia as seen in human restenotic lesions following balloon angioplasty and provide a permissive environment for arterial smooth muscle cell (ASMC) proliferation, migration, and macrophage adhesion. Interference with the association of hyaluronan and versican with the surface of ASMCs, either through short oliogosaccharides of hyaluronan or blocking antibodies to the hyaluronan receptor, CD44, blocks the proliferative and migratory response of these cells to growth factors, such as platelet derived growth factor (PDGF). Agents that interfere with the proliferative response of ASMCs and that are used in the treatment of restenosis, such as rapamycin, inhibit the synthesis of hyaluronan by these cells. Inhibition of versican by versican antisense blocks proliferation of SMCs. The synthesis of hyaluronan and versican is highly regulated and influenced by pro-inflammatory growth factors such as PDGF and transforming growth factor-beta (TGF-beta).

2. INTRODUCTION

The vascular extracellular matrix (ECM) is a reinforced composite of collagen and elastic fibers embedded in a viscoelastic gel of proteoglycans, hyaluronan and water, together with a wide arrangement of assorted glycoproteins (1). These structurally different components interact by entanglement and cross-linking to form a bioactive polymer which, in part, regulates the biomechanical properties of the vasculature and the phenotype of the vascular cells. What is important to realize is that the amount of each of these components is relatively set for any given tissue. For example, most normal large and medium-sized arteries contain large amounts of elastic fiber protein (as much as 50% of the total ECM protein), lesser amounts of collagen, and sparse amounts of proteoglycans and glycoproteins. However, in vascular disease, these proportions change, with proteoglycans predominating in early vascular lesions and collagen found in abundance in later vascular lesions. Proteoglycans and associated ECM molecules influence many of the events associated with the development of the vascular lesions such as lipid retention (2), calcification (3), smooth muscle cell phenotype (4), and retention of macrophages (5).



Figue 1. Sections from stented human coronary artery 11 months after stenting. Lesion can be seen forming on top of the stent wire struts. Arteries were fixed in formalin. The stent wires were carefully removed under a dissecting microscope before processing for light microscopy and paraffin embedding. Sections were then immunostained for versican (A) using a rabbit polyclonal antibody specific for versican, kindly provided by Richard LeBaron (University of Texas at San Antonio, TS). Some sections were immunostained with a polyclonal antibody to biglycan (LF-51) (B) kindly provided by Larry Fisher of the National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD. Other adjacent sections were treated with biotinylated hyaluronan-binding region of aggrecan for the detection of hyaluronan (C) as we have previously described (6). The lesions stain intensely for hyaluronan and versican, but not biglycan. Sections kindly provided by Andrew Farb, Frank Kolodgie, and Renu Virmani..



Figue 2. Sections from human temporal arteries with pseudoaneurysms demonstrating that this vascular lesion is enriched in proteoglycans (Movats stain) (A) and versican (B), but devoid of biglycan (C) and decorin (D). Antibodies and reagents were the same as described above for Figue 1.

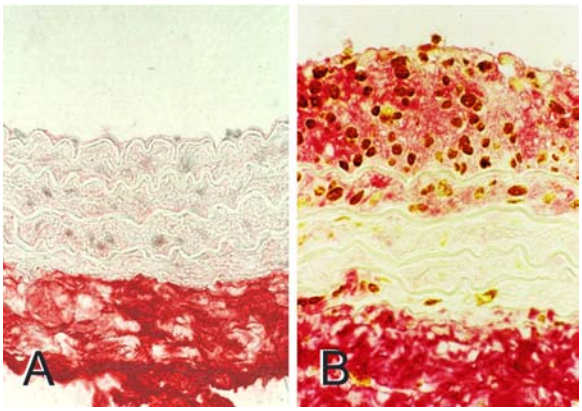
3. VASCULAR PATHOLOGY

Two ECM molecules that are prominent in vascular remodeling associated with vascular disease are hyaluronan and versican. Versican is a high molecular weight chondroitin sulfate proteoglycan that binds to a very large glycosaminoglycan chain of hyaluronan to form large multimolecular aggregates within the ECM of blood vessels and other tissues. These ECM components are enriched in human restenotic lesions that form after balloon angioplasty (6, 7) (Figue 1) and typify the progression of vascular lesions associated with surgical graft vascular repair (9, 10). They are also prominent throughout the ECM in pseudoaneurysms of the human temporal artery (11) (Figue 2). Furthermore, hyaluronan and versican are enriched in advanced human atherosclerotic plaques and accumulate at the plaque thrombus interface suggesting possible roles in the thrombotic process (12, 13). Balloon carotid injury following angioplasty leads to increases in hyaluronan surrounding proliferating arterial smooth muscle cells throughout the intimal layer of blood vessels suggesting a role for these ECM molecules in controlling smooth muscle proliferation (6) (Figue 3).

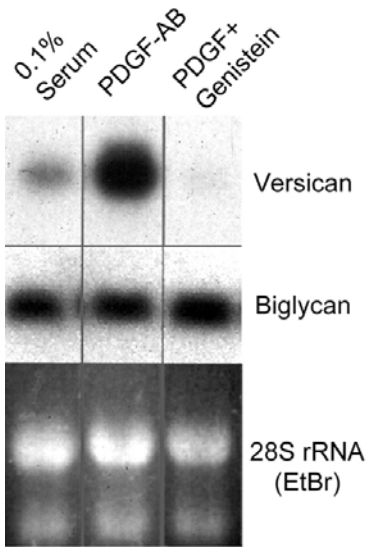
4. REGULATED SYNTHESIS BY ARTERIAL SMOOTH MUSCLE CELLS

A major source of hyaluronan and versican in the vascular wall is the smooth muscle cell (14-17). The versican gene (CSPG2) and protein follow a domain template. An amino-terminal globular domain (G1) binds hyaluronan while the carboxy terminal domain (G3) consists of a C-type lectin adjacent to two epidermal growth factor domains and a complement regulatory region. The middle region of the versican core protein is encoded by two large axons that specify the chondroitin sulfate attachment regions of versican. RNA splicing occurs in the two large axons encoding the GAG attachment domains giving rise to four spliced variants of versican (18). The synthesis of versican is highly regulated and involves regulation of the promoter region of the gene through a beta-catenin-T-cell factor complex in vascular smooth muscle cells (19). On the other hand, hyaluronan is a glycosaminoglycan with no protein component and composed of repeating disaccharide units of n-acetyl-D-glucosamine-β (1→4) -D-glucuronic acid-β (1→3). Synthesis of hyaluronan takes place at the plasma membrane by one or more of three hyaluronan synthases (termed HAS-1, 2, and 3) (20). The hyaluronan synthase gene has a number of transcription factor binding sites involved in inflammatory reactions including NFκB (21).

The synthesis and secretion of hyaluronan and versican are regulated by growth factors such platelet-derived growth factor (PDGF) and transforming growth factor-beta1 (TGFbeta1) (17, 22, 23) (Figue 4). Interestingly, other inflammatory cytokines such as IL-1beta have opposing effects on each of these two molecules, namely IL-1beta stimulates hyaluronan production by arterial smooth muscle cells while inhibiting versican synthesis (25). While increased synthesis undoubtedly contributes to extracellular matrix build up of these macromolecules within developing vascular lesions, less in known about their state of degradation and turnover. Hyaluronan can be degraded by pathways that involve oxidation through generation of free radical damage. In addition, hyaluronan can be cleaved by specific enzymes either present within lysosomes (Hyal 1) or at the cell surface (Hyal 2). It is clear that fragments of hyaluronan



Figue 3. Sections from a normal rat carotid artery (left panel) and from a rat carotid artery 7 days after balloon injury. Sections were stained for PCNA (proliferative cell nuclear antigen) and for hyaluronan. Since formalin fixation attenuates immunostaining for PCNA, only specimens fixed in methanol were used for double staining for hyaluronan (red) and PCNA (brown). The antibody for PCNA was a mouse monoclonal (Clone PC10, Signet). Staining for hyaluronan was accomplished using biotinylated hyaluronan binding region of aggrecan as described by Riessen *et al* (6). Note the dramatic increase in hyaluronan surrounding PCNA positive cells in the artery following injury.



Figue 4. Northern Blot analyses of PDGF stimulation of proteoglycan synthesis in the presence or absence of genestein, a tyrosine kinase inhibitor in arterial smooth muscle cells. Total RNA was prepared by the single step method of Chomczynski and Sacchi (24). Versican cDNA (C7) was a kind gift of E. Ruoslahti, La Jolla, CA, and biglycan cDNA was provided by Marian Yound and Larry Fisher of the National Institute of Dental and Craniofacial Research, NIH, Bethesda, MD. Probes were ^{32}P-labelled and hybridized as described (22). Notice that PDGF specifically upregulates versican mRNA while having no effect on biglycan (23).

have biologic activity (26), but little is known as to how these fragments contribute to vascular disease. Versican, on the other hand, is degraded by a number of proteases including plasmin (9) and the ADAMTS family of proteases such as ADAMTS 1, 4, and 5 (27, 28). Interestingly, breakdown of versican by ADAMTS-4 and -5 appears to reverse the development of vascular lesions (9).

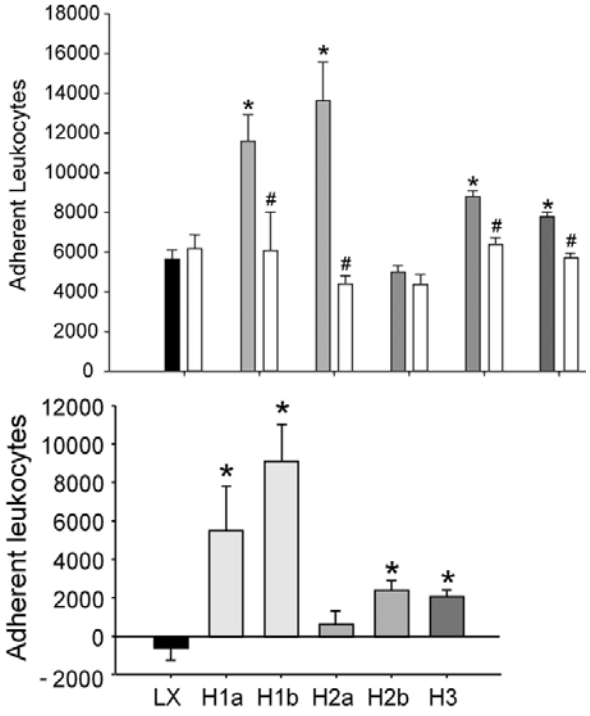
5. ROLE IN PROLIFERATION AND MIGRATION OF VASCULAR SMOOTH MUSCLE CELLS

Hyaluronan and versican form a pericellular ECM around the smooth muscle cells and interference with the formation of this pericellular matrix by short oligosaccharides of hyaluronan blocks the PDGF stimulation of proliferation and migration of these cells (4). Interference with versican synthesis by antisense also inhibits the proliferation of arterial smooth muscle cells (27). Such studies highlight the importance of specific components of the microenvironment surrounding cells in the control of cell phenotype.

6. LEUKOCYTE ADHESION AND RETENTION

In addition to hyaluronan binding to the surface of smooth muscle cells, hyaluronan also binds leukocytes and may be important in causing leukocytes to build up within the vascular wall during the inflammatory phases of vascular disease. For example, hyaluronan is often found in regions of atherosclerotic lesions that contain macrophages (30). Transduction of arterial smooth muscle cells with hyaluronan synthase cDNA creates an ECM the binds monocytes in a hyaluronan-dependent manner (5). The degree of monocyte binding varies depending upon which hyaluronan synthase is expressed with hyaluronan synthase I expression causing the greatest monocyte adhesion to the ECM generated by these cell (Figue 5). The importance of versican in this event is highlighted by the ability of versican antibodies to block monocyte adhesion to this hyaluronan-enriched ECM. Interestingly, agents that interfere with hyaluronan synthesis such as rapamycin (31) also block monocyte adhesion to ECMs synthesized by ASMCs, suggesting that this antimitogenic drug used in the treatment of restenosis might have added anti-inflammatory benefit. Thus, controlling the hyaluronan and versican content of developing vascular lesions may limit the inflammation associated with the development of the disease.

Furthermore, recent studies indicate that angiogenesis, i.e., formation of new vessels throughout the atherosclerotic lesion, is critical to determining the severity of the atherosclerotic plaque (32, 33) and a number of studies have highlighted a role for the ECM in regulating angiogenesis. For example, work by Kumar and Slevin, reviewed in (34), convincingly demonstrates that small fragments of hyaluronan promote endothelial phenotypic change and new blood vessel formation although the precise mechanism responsible for this activity is not known. These studies illustrate that specific components of the ECM may be possible therapeutic targets in the treatment of vascular disease.



Figue 5. Extent of monocyte (U 937) cells binding to an ECM generated by arterial smooth muscle cells transduced with HAS-1, HAS-2, or HAS-3 cDNA. Arterial smooth muscle cells were seeded in 96-well plates at 7 x 10^3/cm^2 and allowed to grow for 6 days. A human monocyte cell line (U937) was labeled with calcein-AM and added to the ASMC monolayers which had been treated with *Streptomyces* hyaluronidase or buffer without enzyme for 30 minutes prior to adding the monocytes. The monocytes were allowed to adhere for 90 minutes at 4ºC. LX = control; LH1a and LH2a = two different clones expressing HAS-1; LH2a and LH2b = two different clones expressing HAS-2; LH3 = one clone expressing HAS-3. Solid bars show amount bound in the absence of pretreatment with *Streptomyces* hyaluronidase and shaded bar shows amount of monocyte adherence when cultures were pretreated with the enzyme. Inset shows only the hyaluronan-dependent adherence of the monocytes. These results show that monocytes adhere to a hyaluronan-enriched ECM generated by arterial smooth muscle cells (5).

7. PERSPECTIVE

Thus, it becomes clear that ECM remodeling involving specific ECM components contribute to vascular disease progression and that such changes may have a multitude of effects on the behavior of the vascular cells. Two specific ECM molecules, hyaluronan and versican play key roles in regulating these phenotypic changes. Testimony to the importance of these molecules in vascular disease is the recent identification of versican as a candidate gene that is altered in a select family of patients with intracranial aneurysm in cerebrovascular disease (35). Such findings indicate that common pathways may exist within ECM in both cerebrovascular and cardiovascular disease. These new clinical findings will need to be further explored.

8. ACKNOWLEDGEMENTS

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