Cell Biology of Arterial Proteoglycans

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Although proteoglycans constitute a minor component of vascular tissue, these molecules have been shown to influence a number of arterial properties such as viscoelasticity, permeability, lipid metabolism, hemostasis, and thrombosis. A hallmark of early and late atherosclerosis is the accumulation of proteoglycans in the intimal lesions. Yet, it is not clear why this accumulation occurs. This article reviews the classes of proteoglycans synthesized by the two major cell types of the arterial wall—the endothelial and smooth muscle cell. Detailed consideration is then given to the modulation of proteoglycan metabolism and the role that proteoglycans play in a number of cellular events such as adhesion, migration, and proliferation—important processes in both the development and the pathogenesis of blood vessels. Last, the involvement of proteoglycans in two critical vascular wall processes—hemostasis and lipid metabolism—is reviewed, because these events pertain to atherogenesis.

This review emphasizes the importance of proteoglycans in regulating several key events in normal and pathophysiological processes in the vascular tissue. (Arteriosclerosis 9:1–20, January/February 1989)

Proteoglycans are a group of complex and diverse macromolecules that are present in all tissues and synthesized by all cells. These macromolecules are found in four locations: 1) throughout the extracellular matrix (ECM), 2) associated with specialized structures of the ECM such as basement membrane and basal laminae, 3) part of, or associated with, the plasma membrane of cells, and 4) in intracellular structures such as secretory storage granules and synaptic vesicles. Proteoglycans contain core proteins to which one or more glycosaminoglycans are covalently attached through O-glycosidic linkage to serine residues in the protein core. Usually one type of glycosaminoglycan predominates on a single core protein, giving rise to four main families: chondroitin sulfate proteoglycan (CSPG), dermatan sulfate proteoglycan (DSPG), heparan sulfate proteoglycan (HSPG), and keratan sulfate proteoglycan (KSPG). In addition to glycosaminoglycans, both N- and O-linked oligosaccharides may be covalently attached to the protein core. Figure 1 shows a schematic representation of the basic structure of a typical proteoglycan.

The diversity of proteoglycans largely derives from the number of different core proteins within specific proteoglycan families and from the polydispersity produced by a large variety of posttranslational modifications required to construct the final molecule. Figure 2 gives examples of the diversity and structure of typical proteoglycans found in tissues such as blood vessels. No doubt this structural diversity contributes to a variety of different biological functions attributed to these molecules. Recent reviews consider the structural characteristics of proteoglycans in detail.1,2

Although proteoglycans constitute a minor component of vascular tissue (2% to 5% by dry weight) as compared to cartilage (50% by dry weight), a number of studies over the years have demonstrated that these macromolecules are of enormous importance in influencing such arterial properties as viscoelasticity, permeability, lipid metabolism, hemostasis, and thrombosis.3,4,5 To date, three major families of proteoglycans have been identified in blood vessels and synthesized by vascular cells. These families include proteoglycans enriched in chondroitin sulfate, dermatan sulfate, and heparan sulfate. Histochemical,9–11 immunocytochemical,12–17 and biochemical18 data indicate that these different families are distributed differently in the arterial wall (Figures 3, 4, and 5). For example, a large CSPG (−1.2 × 10⁶ M) is present principally in the interstitial matrix of the ECM,9,11,10,11,14,15 while a small DSPG (−120 to 180 kDa) is confined to the periphery of collagen fibrils9,11,14 (Figures 5B and 5C). Heparan sulfate proteoglycans appear to be present in arterial basement membranes13,17 (Figure 5A) and closely associated with elastic fibers. Cell culture studies of vascular cells also have revealed that certain classes of proteoglycans are hydrophobic and may be associated with plasma membranes.19–22 The fact that proteoglycans are found in different locations in the arterial wall indicates probable differences in the function of each family of vascular proteoglycans.

A hallmark of early and late atherosclerosis is the accumulation of proteoglycans in the intimal lesions (Figures 3 and 4).3,4,5,10,22 Such accumulation may predis-
pose the arterial wall to lipid accumulation, calcification, and thrombosis by virtue of the ability of proteoglycans to interact with component molecules involved in these processes. In addition, enrichment in proteoglycans characterizes vascular conditions such as aneurysms associated with Marfan Syndrome, veno-occlusive disease occurring in arteriovenous fistulas, and cerebral amyloid angiopathy, a condition affecting cerebral vessels in Alzheimer's disease. It is of interest that an antibody raised against an arterial CSPG has been used effectively to "image" plaques in developing atherosclerotic arteries in experimental animals. These studies collectively indicate the involvement of proteoglycans in cardiovascular diseases. However, it is still not clear why proteoglycans accumulate and what specific effects these molecules have on events associated with the pathogenesis of these diseases. This review focuses on these two questions with emphasis on recent developments in the field of arterial proteoglycans, since studies prior to 1980 have already been reviewed.

Vascular Cell Proteoglycans

Endothelial Cells

Vascular endothelial cells synthesize and secrete both heparan sulfate and dermatan sulfate containing proteo-
Figure 3. Light micrograph illustrating that the narrow intima of a normal blood vessel stains more intensely with (A) alcian blue and (B) a monoclonal antibody against aortic CSPG than the underlying medial layer. Vessels undergoing intimal hyperplasia (early atherosclerosis) possess thickened intimas that also stain intensely with alcian blue (C) and a monoclonal antibody against aortic CSPG (D). ×281. Reproduced with permission from Wight et al. In: Wight TN, Mecham RP, eds. Biology of proteoglycans. Orlando, FL: Academic Press; 1987:267–300. Bar=50 μm.

glycans. These molecules differ in size and in ability to interact with other molecules. For example, there are a number of size classes of HSPG present in endothelial cultures. One form is hydrodynamically large (~400 kD) and sediments in cesium chloride at low buoyant density. It appears that the larger species of HSPG exists as disulfide bonded aggregates, linked either to itself or to other matrix components, as has been demonstrated for HSPG in a mouse tumor basement membrane. In addition, a HSPG (~250 to 400 kD) from cultured vascular endothelial cells that exhibits hydrophobic properties interacts specifically with antithrombin III and, therefore, is thought to play a critical role in providing a nonthrombogenic surface for the vascular endothelium (see also Hemostasis section).

Structural differences among the subclasses of endothelial derived HSPGs may arise in part from the synthesis of unique HSPGs on cores that are distinct products of separate genes. For example, Slow and coworkers have shown that antibodies raised against a putative hepatocyte plasma membrane HSPG failed to stain glomerular basement membrane, staining only the surfaces of kidney epithelia and endothelia, while the reverse was true for an antibody raised against HSPG from basement membrane. The most recent study by this group has shown that certain cell types in culture synthesize two forms of HSPG, one that possesses hydrophobic properties consistent with a membrane location and another that is nonhydrophobic and reacts only with the antibasement membrane HSPG antibody. Others have shown similarly that structurally distinct cell surface and basement membrane associated HSPGs found in mouse mammary epithelial cells also are immunologically distinct.

A recent study of the high- and low-density forms of HSPG in the Engebrich-Holm-Swarm (EHS) basement membrane producing tumor indicates that the two forms contain distinctly different core proteins and glycosaminoglycan chains. Alternatively, posttranslational or postsecretional processing also may generate some of the multiple HSPG species, either as a mechanism for targeting HSPGs with different functions to different cellular and extracellular compartments or as a result of
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HSPG turnover. For example, Ledbetter et al. identified a 400 kD precursor protein that was converted to a large, low-density HSPG in the EHS tumor system. Similarly, Klein et al. recently presented evidence that the glomerular basement membrane HSPG (M_r = 130,000 to 200,000) was derived from a larger HSPG precursor (M_r = 400,000 to 600,000). Other studies following the secretion, uptake, and intracellular degradation of single HSPG species indicate that several species of HSPG are generated by turnover mechanisms operating in cell culture. Thus, it appears that endothelial cells are the source of a variety of HSPG subclasses. Their origin and specific function(s) await further study.

Vascular endothelial cells also synthesize a family of proteoglycans that contain dermatan sulfate. These proteoglycans contain glycosaminoglycan chains of low ionic acid content (~10%) when the cells are cultured on plastic, but a higher percentage of iduronic acid when cultured on collagen gels. At least three separate sub-species have been identified. One species appears to be a long-lived (t_1/2 = 40 hours) high molecular weight complex consisting of DSPG covalently linked to fibronectin via transglutaminase cross-linking. Two other DSPGs can be distinguished in these cultures (M_r = ~220,000 and ~130,000); these resemble similar subclasses that have been designated as DSPG I and II in cartilage and a variety of other tissues (reviewed in references 56 and 57).

It is now clear that a significant proportion of the proteoglycans synthesized by endothelial cells are deposited as basement membranes. For example, an antibody raised against the large low density HSPG in the EHS tumor immunostains the matrix synthesized by cultured bovine aortic endothelial cells and vascular basement membranes. The role of proteoglycans as part of this specialized structure appears to be at least twofold. One role is to serve as a structural organizer of the other components of the basement membrane (such as type IV collagen, laminin, entactin, and fibronectin) by virtue of the ability of proteoglycans to interact at specific sites within each of these molecules. Another role is to contribute to the selective filtration barrier function of basement membrane by conferring a network of fixed charges to this structure. Alterations in the proteoglycan content of basement membranes severely compromise the functioning of this filtration barrier.

Basement membranes in diabetic tissues have a reduced HSPG content and are undersulfated. Such alterations are thought to contribute to the increased vascular wall permeability frequently seen in diabetes.

**Smooth Muscle Cells**

Unlike endothelial cells that synthesize predominantly HSPGs, vascular smooth muscle cells synthesize and secrete principally CSPGs and DSPGs with considerably less HSPG (Figures 6 and 7). In general, these proteoglycans resemble the bulk of the proteoglycans present in intact arteries. Chondroitin sulfate proteoglycan is a major arterial proteoglycan. Biochemical and ultrastructural studies of CSPG synthesized by cultured monkey arterial smooth muscle cells indicate that they are hydrodynamically large molecules (M_r = 1,2 x 10^6) that consist of a core glycoprotein measuring 220 nm to 320 nm in length (M_r = 390,000 to 500,000) to which are attached 15 to 20 chondroitin sulfate (CS) chains with M_r ~ 40,000 to 60,000. In addition, a large number of O-linked oligosaccharides (~300) and a few N-linked oligosaccharides are attached to the CS core protein. A similar size core glycoprotein has been observed from CSPG isolated from bovine aorta after removal of the CS chains by chondroitinase digestion and the presence of both N- and O-linked oligosaccharides on a large CSPG isolated from human aorta has been confirmed. The large CSPG isolated from blood vessels and arterial smooth muscle cell cultures aggregates with hyaluronic acid (HA) and in this respect resembles the major and well-characterized CSPG present in cartilage. However, Hainevård et al. recently demonstrated that the large aortic CSPG, while containing the HA binding region, gave different peptide maps and showed only partial homology when compared to the aggregating CSPG in cartilage, suggesting differences in the core proteins of these two CSPGs. The finding of a single species of link protein present in blood vessels suggests that, like cartilage, the HA-CSPG...
aggregate formed in blood vessels is stabilized by at least one accessory link protein.

Immunocytochemical studies reveal that a large CSPG is restricted to the interstitial matrix of arterial smooth muscle cell cultures and blood vessels and is not part of other matrix components such as collagen, elastic fibers, and basal laminae. 

However, a smaller CSPG that has hydrophobic properties and is immunologically distinct from the large interstitial CSPG is present in nonhuman primate arterial smooth muscle cell cultures and may be associated with the plasma membrane of these cells. A CSPG has been identified on the surface of rat arterial smooth muscle cells, as well as associated with membranes of other cell types. At present, it is not clear what role these membrane-associated proteoglycans play in the physiology of vascular cells, but they most probably are involved in mechanisms associated with cell adhesion, migration, and proliferation (see next section).

A second population of smaller proteoglycans containing dermatan sulfate also is synthesized by arterial smooth muscle cells. Electron microscopy of purified DSPG from these cells reveals a short core glycoprotein (100 nm) with 1 to 2 glycosaminoglycan side chains of Mr \( \approx 40,000 \) to 60,000 (Figure 2). The core glycoprotein of this class is \( \approx 45,000 \) (Figure 7). These cells appear to synthesize two types of DSPG. One species is present in the culture medium and contains a low percentage of iduronic acid (<20%). Another class is deposited in the cell layer matrix and contains a much higher percentage of iduronic acid (~50%); it is the predominant proteoglycan deposited when arterial smooth muscle cells are cultured on collagen gels. Two types of DSPG that vary in iduronic acid content also have been found in intact blood vas-
**Figure 6.** Gel filtration chromatography of $^{35}S$-radiolabeled arterial smooth muscle cell layer extracts at quiescence (A), during growth stimulation (B), or grown in the presence of collagen gels (C). In A, peak I contains a mixture of two hydrophobic proteoglycans, one that contains heparan sulfate chains and one that contains chondroitin sulfate (CS) chains. Peak II contains chondroitin sulfated proteoglycan and Peaks III and IV contain dermatan sulfate proteoglycans. Inset in B represents total incorporation of $^{35}S$SO$_4$ into proteoglycan expressed on a per cell basis, at quiescence (open bar) or growth stimulation (shaded bar). Inset in C represents similar radioactivity from cells on plastic (open bar) or within collagen gels (shaded bars).

**Figure 7.** Core glycoprotein analysis of the two major classes of proteoglycans synthesized and secreted by arterial smooth muscle cells. A. $^{35}$S-methionine radiolabeled proteoglycans were immunoprecipitated with a monoclonal antibody against aortic CSPG and electrophoresed in a 3% to 7% SDS-polyacrylamide gel, Lane 1. The immunoprecipitate was digested with chondroitinase AC for 3 hours and was electrophoresed (Lane 2). The three high molecular weight bands represent core glycoproteins devoid of GAG chains. $^{35}$S$^3$O$_4$-radiolabeled chondroitin sulfate proteoglycan (CSPG) isolated from a Sepharose CL-2B column, ($K_w$=0.31) was electrophoresed in Lane 3, and $^{35}$S-methionine labeled proteoglycan was incubated with an antidermatan monoclonal antibody as a control (Lane 4). B. Illustrates the electrophoretic behavior of dermatan sulfate proteoglycans (DSPG) synthesized by arterial smooth muscle cells. Lane 1 contains $^{35}$S-methionine labeled material Immunoprecipitated with a polyclonal antibody to DSPG. Lane 2 represents the immunoprecipitate after chondroitinase ABC treatment.

Dermatan sulfate proteoglycans synthesized by arterial smooth muscle cells resemble similar molecules found in a number of tissues including sclera, tendon, skin, cartilage, blood vessels, and blood vessels. In vitro translation experiments with RNA from arterial smooth muscle cells and an antibody against the small DSPG present in cartilage and skin indicates that the core protein of the arterial smooth muscle cell DSPG is $\sim 41$ kDa and is immunologically related to both skin and cartilage DSPG. In addition, recent experiments indicate similar core protein sizes for DSPGs that are synthesized by arterial smooth muscle cells and skin fibroblasts. Interestingly, however, the DSPG synthesized by arterial smooth muscle cells is larger, due to longer glycosaminoglycan chains, and contains less iduronic acid than the DSPG secreted by skin fibroblasts.

Both the large (CSPG) and the small (DSPG) proteoglycans synthesized by arterial smooth muscle cells in culture appear to be secreted at similar rates with a $T_{1/2}$ of $\sim 8$ minutes (i.e., transit time from Golgi to the cell surface).
Table 1. Arterial Smooth Muscle Cell

| Type  | Size* (Kav) | Characteristic          | Location                        |
|-------|-------------|-------------------------|---------------------------------|
| CSPG (1) | 0.31       | aggregating             | interstitial matrix             |
| CSPG (2) | 0.60       | hydrophobic             | plasma membrane (?)             |
| DSPG (IdA) | 0.65      | iduronic acid-rich      | collagen                        |
| DSPG (GIA) | 0.65      | glucuronic acid-rich    | ?                               |
| HSPG (1)  | 0.60       | hydrophobic             | plasma membrane                 |
| HSPG (2)  | 0.60       | detergent-resistant     | matrix, basement membrane (?)   |

*Hydrodynamic size (elution position) on a Sepharose CL-2B column (4M GuHCl)

CSPG—chondroitin sulfate proteoglycan, DSPG—dermatan sulfate proteoglycan, HSPG—heparan sulfate proteoglycan.

(Yeo and Wight, unpublished observations), which is comparable to the secretion kinetics of other proteoglycan secreting cells such as cartilage chondrocytes, skin fibroblasts. However, it is of interest that these two types of proteoglycans are internalized by arterial smooth muscle cells at different rates, with DSPG exhibiting rapid, high-affinity, and saturable kinetics of uptake, whereas CSPG is taken up more slowly and by a low-affinity endocytic process. These data indicate that the turnover of different families of proteoglycans proceeds at different rates.

A third class of proteoglycan synthesized by arterial smooth muscle cells contains heparan sulfate chains. This class represents a minor fraction of the total proteoglycans synthesized by these cells and is usually found in larger amounts in the cell layer matrix (~20%) than in the medium (<5%). The majority of HSPG present in the cell layer elutes as a complex in the void volume of a Sepharose CL-2B column but shifts to an included position of Kav 0.6 when extracted in the presence of detergent. The HS chains are long (M̄, ~40 000 to 60 000) and both N- and O-linked oligosaccharides are attached to the protein core. This molecule has an affinity for hydrophobic binding resins and may represent an intercalated plasma membrane form of HSPG. Recent studies have identified a population of HSPGs on the surface of rat arterial smooth muscle cells that gives rise to heparan sulfate chains upon trypsin treatment of the cultures. In addition to the hydrophobic HSPG, arterial smooth muscle cells synthesize a nonhydrophobic HSPG of similar size. At this point, it is not clear how these two forms are related and whether they represent distinctive populations specifically associated with plasma membranes and ECM structures such as basal laminae.

The above studies illustrate that multiple subclasses of proteoglycans exist within each family of arterial proteoglycan. Table 1 presents a tentative list of the various subclasses of arterial smooth muscle cell proteoglycans. It now becomes important to determine whether these subclasses have distinctly different biological functions.

Cell Adhesion

The capacity of endothelial cells to adhere to their connective tissue substrata is critical to the maintenance of normal vascular homeostasis. In fact, temporary detachment of endothelial cells has been postulated as one of the early events in the genesis of atherosclerotic lesions. Experimental removal of endothelial cells leads to marked atherosclerotic development in experimental animals. Thus, it is important to understand those factors involved in endothelial cell adhesion. Proteoglycans have been implicated in the adhesion of a variety of cells to their substratum. Most cells, including vascular endothelial cells, interact with their substrata in specialized areas of the cell known as focal adhesion sites. These sites are enriched on the ECM side in attachment proteins, such as fibronectin and heparan sulfate proteoglycans, and on the cytoplasmic side in actin microfilaments. Colocalization of HSPG with cytoskeletal elements such as actin suggests that proteoglycans may influence cell adhesion through association with the cytoskeleton. Recent studies have shown that the integrity of actin stress filaments is critical for endothelial cell adherence when exposed to shear stress. Although it is not completely certain as to how cell-associated HSPG mediates cellular adhesion, one theory is that proteoglycans may stabilize the adhesion process by interacting with specific proteoglycan binding domains in the attachment protein, such as fibronectin, thus stabilizing the interaction of fibronectin with its cell surface receptor. It is of interest that removal of HSPG from endothelial cells does not detach cells but does prevent cells from reattaching. Such studies indicate that endothelial cells, like many other cell types, possess multiple adhesion mechanisms. The fact that heparin and heparan sulfate treatment of deposited endothelial-derived extracellular matrix significantly inhibits endothelial cell adhesion supports the hypothesis that proteoglycans may be facilitating attachment by interacting with other adhesion molecules. Late passage endothelial cells that detach more readily than early passage endothelial cells also are characterized by less proteoglycan at their attachment sites.

While HSPG appears to promote cell attachment for some cells, other proteoglycans have opposite effects. For example, both CSPG and DSPG inhibit the attachment of a variety of cells to either fibronectin or type I collagen. These proteoglycans may destabilize focal cellular adhesions, possibly interfering with specific cell binding sites in the adhesive glycoproteins. For example, the proteoglycan may bind to the adhesion protein and sterically interfere with the binding of the protein to its cell surface receptor. Alternatively, the binding of proteoglycan may change the configuration of the protein, causing a decrease in its association constant for the receptor. Evidence for a conformational change upon proteoglycan binding has recently been reported for plasma fibronectin. Thus, it appears that proteoglycans influence adhesion by modulating the activities of the primary molecular components involved in this process.

Proteoglycans also may influence cell-to-cell association. For example, the heparan sulfate chains of HSPG enriched in iduronic acid from postconfluent fibroblasts show selective affinity for cognate heparan sulfate...
chains. This property may enable membrane forms of HSPG to self-associate and thus have a stabilizing influence on cell-cell association. Growing cells, which should be less adhesive, contain cell-surface HSPG with heparan sulfate chains that show little tendency to self-associate. Proteoglycans also appear to induce the formation of gap junctions in primary liver cultures by simulating the synthesis of a specific gap junction protein. It remains to be seen whether a similar situation exists for other types of cells such as vascular endothelial and smooth muscle cells.

Cell Migration

The migration of vascular endothelial cells in response to injury or during angiogenesis and of smooth muscle cells during intimal hyperplasia are critical events in vascular wall development and disease. The migration of vascular cells is thought to be highly dependent on their ability to synthesize and secrete certain components of the extracellular matrix. For example, Matr and Silen have shown that changes occur in the distribution of collagens and laminin during vascular endothelial cell sprouting and migration in vitro and have suggested that continued secretion of collagen is required for effective migration of these cells. Kinsella and Wight have demonstrated that endothelial cells induced to migrate by wounding increase their synthesis of proteoglycan by fourfold and shift from synthesizing a primarily HSPG-rich endothelial matrix to synthesizing a CSPG/DSPG-rich matrix (Figure 8). Autoradiographic evidence revealed that incorporation of proteoglycan precursor (35S-sulfate) was greatest in migrating cells near the wound edge. These results support the concept that proteoglycans participate in the migratory response of cells. A number of recent studies indicate that the migration of neural crest cells is inhibited by the addition of CSPG when added to collagen or fibronectin matrices, suggesting decreased cell-substratum adhesion. It is tempting to speculate that the glucuronic acid-rich DSPG synthesized by endothelial cells after wounding is involved in the facilitation of migration by interfering with the cell's adhesive mechanism. Oohira et al. noticed the loss of HSPG in cultures of sprouting (migrating) endothelial cells and Ausprunk et al. presented histochemical evidence suggesting that HSPG is depleted at the tips of growing capillaries. Other factors known to influence angiogenesis, such as interleukins, also exhibit a marked effect on proteoglycan deposition by vascular endothelial cells, but it is not known whether such factors influence the migration of these cells.

Smooth muscle cell migration from the media to the intima of blood vessels is an important component of the early vascular response to injury and, as such, may play a pivotal role in atherosclerosis. Little is known about the factors that control this process. Recent studies by Majack and Cowes have shown that heparin inhibits arterial smooth muscle cell migration in vitro in a dose-dependent and reversible fashion, but hyaluronate and chondroitin or dermatan sulfate do not. Furthermore, this effect was specific for arterial smooth muscle cells, since similar treatments had no effect on cultured bovine aortic endothelial cells or Swiss 3T3 cells. In fact, heparin stimulates the migration of bovine capillary endothelial cells. The mechanism for the antimitogenic effect of heparin on smooth muscle cells is not yet known.

Such studies indicate that vascular cell migration is accompanied by qualitative and quantitative changes in proteoglycans. It remains to be shown whether such changes regulate this cellular response and if so, how these changes affect the "migratory machinery" of the cell.

Cell Proliferation

Two key events in the development of the atherosclerotic plaque are the proliferation of arterial smooth muscle cells and the deposition of components of the extracellular matrix. An important question is whether these two events are related. Recent studies indicate that quiescent arterial smooth muscle cells increase their synthesis of proteoglycan when stimulated to divide (Figure 6B) and this increase occurs principally during the G1 phase of the cell cycle. The activity of several enzymes involved in the synthesis of chondroitin sulfate chains,

![Figure 8. A. Time course of 35S-sulfate incorporation into proteoglycan after multilayer wounding of confluent endothelial monolayer cultures. Maximum incorporation (proteoglycan synthesis) is observed at 44 to 50 hours at the time when endothelial cell migration is maximal. B. Shaded areas represent proportion of total radioactivity present in the cell layer. B. DEAE-Sephacel on exchange chromatography of 35SO4-labeled cell layer extracts from confluent (- -) and wounded (---) cultures 48 hours after wounding. Note the large increase in the proportion of radioactivity associated with DSPG containing peak C.](image-url)
such as xyllosyl transferase, N-acetylglactosaminyl transferase I, and two sulfotransferases, increase during the proliferative phase in arterial smooth muscle cells. These results suggest that stimulation may involve, in part, an increased capacity of the cells to synthesize the glycosaminoglycan moiety of the proteoglycans. This increase in synthesis is not restricted to proteoglycans, since other matrix molecules such as collagen and thrombospondin also increase during growth stimulation. These changes may, in part, account for the initial accumulation of matrix molecules during the proliferative phase of atherosclerosis. At present, it is not clear whether these synthetic modulations are necessary for proliferation or are a consequence of the proliferative response. It is of interest that growth stimulation of density-arrested Balb 3T3 cells is accompanied by an increase in the capacity of the cells to glycosylate proteins, and interference with this capacity markedly inhibits some cells from progressing through their cell cycle. Such results suggest that cell cycle progression may rely on the expression of specific glycoproteins and/or proteoglycans.

Proteoglycans also have been implicated in the inhibition of cell proliferation. For example, HSPG derived from postconfluent endothelial and smooth muscle cells inhibits the proliferation of arterial smooth muscle cells, and this inhibitory activity is highly dependent on the presence of a platelet h proved that 148 and this enzyme generates dodecasaccharide heparan sulfate fragments, which are active as growth inhibitors. Similar heparinase activity is also present in resident cells of the arterial wall, including smooth muscle cells as well as blood-derived cells such as lymphocytes, macrophages, and neutrophils. In fact, extravasation of blood cells into the arterial wall is correlated with levels of heparinase activity. The fact that lymphocytes, macrophages, and neutrophils are present in lesions of atherosclerosis indicates a potential for the generation of proliferation-inhibitory proteoglycan fragments in vascular disease. Smooth muscle cells possess specific high-affinity receptors for heparin/heparan sulfate and can internalize this bound glycosaminoglycan. These results raise the interesting possibility that heparin/heparan sulfate and heparin/heparan sulfate fragments may suppress the growth of smooth muscle cells at specific intracellular sites. Additional studies support this possibility. For example, HSPG is present on the hepatocyte cell surface as a phosphatidylinositol-linked proteoglycan. This HSPG is metabolically processed on the cell surface with the loss of the diacylglycerol portion of the phosphatidylinositol and is internalized by receptor-mediated endocytosis. A part of the HSPG is then processed in the nonlysosomal compartment of the cell, and selected fragments of heparan sulfate chains containing an unusual sulfated glucuronic acid appear in the nucleus without the core protein. Changes in the structures and amounts of the nuclear heparan sulfate are accompanied by changes in the growth behavior of hepatocytes. Such studies indicate that the internalization of HSPG by a cell may, in part, regulate the growth of that cell.

Thus, evidence continues to accumulate that some forms of HSPG act as growth modulators, yet the precise mechanism(s) is still not known. A number of studies have shown that heparin alters the secretory phenotype of cultured arterial smooth muscle cells. For example, heparin induces the synthesis of a 50,000 molecular weight collagen-like protein at 18 to 24 hours after the addition of the heparin molecule. Other proteins of lower molecular weight (i.e., 35, 37, 39 kd), believed to be apolipoprotein (apo) E containing proteins, are induced within 4 to 8 hours of heparin exposure. No direct correlation between heparin regulation of these proteins and growth inhibition has been established, but it is noteworthy that these synthetic changes occur at dosages that are normally growth-inhibitory.

A recent finding that heparin decreases thrombospondin concentration in the extracellular matrix of cultured arterial smooth muscle cells is of interest because antibodies to thrombospondin were found to inhibit DNA synthesis in these cells. In addition, thrombospondin and the mitogen epidermal growth factor (EGF) act synergistically to stimulate DNA synthesis by smooth muscle cells, and this effect is inhibited by heparin. Thus, it may be that certain components of the extracellular matrix are necessary for cellular proliferation and that other matrix components, such as proteoglycans, influence the availability of such molecules. The fact that cell-associated HSPG can serve as a receptor for thrombospondin supports this possibility. It is also possible that heparin-like molecules regulate growth by interfering with the response to specific mitogens. For example, heparin reduces the number of EGF receptors on smooth muscle cells. Thus, the effect of heparin on growth may be due to a combination of effects on the matrix surrounding the cells and on the mitogenic machinery of the cell itself. Heparin also is effective in reducing smooth muscle cell proliferation in vivo after arterial injury and is thus capable of counteracting the effects of local and blood borne growth factors. Associated with this cessation of growth is an increase in the concentration of proteoglycans in injured arteries. In vitro studies have also shown that arterial smooth muscle cell cultures accumulate more proteoglycan in the presence of heparin. The nature of this modulation is currently under study. The reader is also referred to a recent review that discusses heparin's effect on other arterial processes.

Although most of the evidence indicates that the ability of heparin and heparan sulfate to inhibit growth is not caused by their interaction with peptide growth factors to inactivate them, a variety of peptide growth factors with mitogenic activity toward smooth muscle cells, fibroblasts, and endothelial cells display heparin binding affinity. Endothelial cell growth factor (ECGF) is a polypeptide mitogen that has been purified and characterized from bovine neural tissue. This mitogen supports the proliferation and serial propagation of human endothelial cells in vitro. Heparin binds to ECGF and potentiates the mitogenic activity of this polypeptide. The effectiveness of the interaction with heparin correlates with the ability of the mitogen to stimulate endothelial cell proliferation. Heparin also releases ECGF from the surface of cultured endothelial cells. Although the mechanism of heparin action is unclear, the synergistic activity suggests struc-
tural interactions between the carbohydrate and mitogen. It is possible that heparin potentiates the activity of ECGF by causing the mitogen to assume a conformation with increased affinity for its receptor.176 Likewise, it is possible that the receptor requires a heparin-ECGF complex for an "ideal fit." Heparin also exhibits affinity for other growth factors such as platelet-derived growth factor,179 insulin-like growth factor,179 and fibroblast growth factor.190,191,192 Such interactions may effectively compartmentalize and retain growth factors for maximal activity. For example, Vlodavsky and colleagues have shown that heparin-binding FGF-like growth factors, which are angiogenic, are sequestered in the extracellular matrix by virtue of their binding to HSPG.181,182 Destruction of the heparan sulfate chains by heparinase efficiently releases the growth factor from the extracellular matrix of basement membranes.182 Similar sequestration of growth factors by proteoglycan has also been observed in bone marrow.183,184 Collectively, these studies indicate that the glycosaminoglycan heparin, which is derived from mast cells, is capable of potentiating the action of growth factors in vitro. The presence of mast cells at the leading edge of developing blood vessels and the fact that heparin stimulates the growth of endothelial cells in vitro and angiogenesis in vivo suggests that mast cell heparin is important in modulating vascular cell behavior in vivo.185,186 Consistent with this suggestion is the observation that mast cell granules containing heparin can be internalized and degraded by endothelial cells187 and can cause proliferation of microvascular endothelial cells.188 It also may be that heparin-like molecules in the form of HSPG synthesized by endothelial or smooth muscle cells are effective in binding and potentiating the action of these different mitogens as suggested by the studies cited above. As discussed earlier, the HS chains in HSPG have some structural features in common with heparin derived from mast cells. In fact, a recent study demonstrates heparin sequences in the heparan sulfate chains of an endothelial cell proteoglycan.189 Anticoagulant HS has been isolated from vascular tissue that was shown to be free of mast cells.190 However, Imamura and Mitsui191 recently demonstrated that heparin sulfate and heparin exhibited opposing activities on the growth of human umbilical vein endothelial cells in the presence of acidic and basic FGF. Such results illustrate that the two molecules may differ in their activities associated with growth.

Proteoglycans also may act as receptors for growth regulatory substances. For example, Fransson et al.191,192 have shown that the core protein of a cell surface HSPG in fibroblasts binds transferrin and appears to have a structure nearly identical to the transferrin receptor. In addition, thrombospondin binds to the surface of vascular endothelial cells in a receptor-like fashion and this binding is inhibited by heparin, suggesting that thrombospondin associates with the surface of endothelial cells in a HSPG-dependent manner.193 The importance of thrombospondin in regulating smooth muscle cell growth194,195 suggests a growth regulatory role for the HSPG present on the surface of smooth muscle cells. It remains to be determined whether thrombospondin associates with the surface of smooth muscle cells as it does with endothelial cells. Majack et al.196 made the interesting suggestion that surface thrombospondin, presumably bound to a membrane form of HSPG, interact with certain proteases to facilitate the matrix degradation and remodeling that accompanies cell proliferation. Heparin, which inhibits SMC proliferation, also releases thrombospondin from the cell surface. Such loss of thrombospondin may result in dissociation of the protease from the cell surface and loss of the capacity of matrix degradation to permit cell division. Such speculation awaits proof.

Recent studies have shown that the high molecular weight receptor for transforming growth factor beta (TGFβ) (a multifunctional protein that influences growth and connective tissue synthesis in a wide variety of cells) is a proteoglycan containing heparan sulfate and CS chains.197 These studies illustrate that cell surface-associated proteoglycans may play critical roles in the regulation of cell growth and differentiation.

Modulation of Proteoglycan Metabolism

A number of other factors are capable of modulating proteoglycan metabolism by vascular cells in the absence of cell proliferation and migration. For example, TGFβ, a platelet product, stimulates CSGP synthesis by human arterial smooth muscle cells without the stimulation of cell proliferation.198 This effect is cell-specific in that this platelet product had no effect on proteoglycan synthesis by vascular endothelial cells. The type of extracellular matrix surrounding vascular cells also influences the metabolism of proteoglycans. For example, when arterial smooth muscle cells are cultured on hydrated collagen gels (type I collagen), they decrease their overall proteoglycan accumulation (expressed on a per cell basis) but increase the amount of uronic acid-rich dermatan sulfate present in the extracellular matrix (Figure 6C).199 Pulse-chase studies indicate that the accumulation of this specific proteoglycan within the cell layer is due partly to decreased turnover and partly to increased synthesis. Other extracellular factors shown to influence proteoglycan synthesis by cultured vascular cells include oxygen,190,191 prostanoids,192 and lipids.193,194,200 These studies indicate that a variety of factors are capable of influencing proteoglycan metabolism by vascular cells.

Hemostasis

Endothelial cells provide a nonthrombogenic lining for the vascular system by secreting or sequestering factors involved in maintaining blood fluidity such as plasminogen activators,201 prostacyclin,202 and thrombomodulin.203 In addition, endothelial cells synthesize and secrete heparin-like HSPGs that bind to antithrombin III and enhance the inactivation of thrombin, inhibiting the clotting cascade.19,34,37,38,204 Heparin and heparan sulfate are related molecules that are part of a large family of heterogeneous proteoglycans.205,206 Heparin is distinguished from heparan sulfate in that it is more highly charged, appears to occur entirely in mast cells, and is an effective anticoagulant. However, heparin has many structural features in com-
mon with heparan sulfates. Both mast cell heparins and HSPGs contain anticoagulant and nonanticoagulant species.36,37,38,183,204 Generally, heparin contains more N- and O-sulfate and more iduronic acid but less N-acetyl and glucuronic acid than do HSPGs. Heparan sulfates, on the other hand, have a more ubiquitous animal and tissue distribution. Refer to Hovingh et al.225 for a discussion of the relationship between these two sets of molecules.

The binding of heparin and heparan sulfate to antithrombin III occurs at lysyl residues in the antithrombin molecule, and is thought to involve a specific molecular domain composed of the tetrasaccharide sequence: IdA (GlcA)—GlcN-6-O-SCV-GIcA—GlcN-3-O-SO₂ which is absent in nonanticoagulant heparin or heparan sulfate (for reviews, see references 38 and 205). This binding is responsible for a 1000-fold acceleration of enzyme-inhibitor complex formation. It is of interest that other purified hemoestatic enzymes of the intrinsic coagulation cascade, i.e., factors IX, Xa, Xai, and XIIa, also are neutralized by antithrombin III in a similar heparin-dependent way. However, heparin induces only a 4- to 15-fold enhancement in the rate of neutralization of these factors by antithrombin III.36

Recent studies indicate that a very small percentage of the total HSPG synthesized by aortic and microvascular endothelial cells (~1% and 10%, respectively) accounts for the majority of antithrombin III binding18 and anticoagulant activity. This HSPG binds to hydrophobic regions consistent with a membrane location and contains heparan sulfate chains that are enriched in the antithrombin III binding disaccharide sequence of GlcA—GlcNAcSO₂-3-O-SO₂. These findings also demonstrate that molecules synthesized by cells other than mast cells possess anticoagulant properties.

It appears that endothelial surface proteoglycans are capable of binding not only anticoagulant factors but also procoagulant factors.207,208 For example, thrombin binds to the surface of cultured porcine aortic endothelial cells in a rapid and reversible manner and this binding is partially inhibited by pretreatment of the cells with purified heparinase,209 an enzyme that cleaves heparan sulfate chains. Thus, it may be that the inactivation of thrombin by antithrombin III is regulated, at least in part, by one or more HSPGs present at the surface of the endothelial cell and that bind both coagulant and anticoagulant factors.

The role of platelets in hemostasis, thrombosis, and atherosclerosis is well established.103,209,210 Less clear is what specific role(s) platelet secretory products play in arterial wall metabolism. For example, platelets contain a variety of substances that are stored in alpha granules and released when platelets are stimulated to aggregate in tissue injury, promoting coagulation at the damaged site.210 Among these substances are a number of antithrombin factors such as platelet factor 4 (PF4), which binds to heparin and neutralizes heparin activity.212 PF4 occurs complexed to a chondroitin sulfate proteoglycan,211 which appears to function as a carrier molecule. More recent studies indicate that megakaryocytes synthesize at least two forms of CSPG and these forms, which are present within alpha granules of platelets, are released in response to thrombin.212 The significance of the PF4:PG complex in arterial wall metabolism needs to be elucidated. The fact that heparin releases PF4 into the circulation similar to the release of lipoprotein lipase213 suggests that PF4 also binds to a heparin-like molecule on the endothelial cell surface. In fact, additional studies demonstrate that PF4 binds to the surface of cultured human umbilical vein endothelial cells in a time-dependent, saturable fashion and that heparin and HS compete for binding.214

**Lipid Metabolism**

Endothelial cell surface HSPGs also are involved in lipid metabolism. For example, lipoprotein lipase [a dimeric enzyme responsible for the hydrolysis of di- and triacylglycerol constituents of plasma very low density lipoproteins (VLDL) and chylomicrons] interacts with the surface of endothelial cells in a heparin/heparan sulfate-dependent fashion.215,216,217 Recently, Klinger et al.218 used a lipoprotein lipase-agarose affinity column to isolate an HSPG from a mixture of proteoglycans in rat brain and found it to be a membrane-associated proteoglycan of approximately 220 kD containing glycosaminoglycan chains of Mᵤ=14 000 to 15 000. The mechanism by which this proteoglycan interacts with the enzyme is not clear, but most probably involves the glycosaminoglycan portion of the molecule, since glycosaminoglycan chain length and the number of N-sulfate and N-acetyl groups on the glycosaminoglycan chain affect affinity for lipoprotein lipase.219,220

Another way in which endothelial cell-derived proteoglycan may modulate lipid metabolism is by modifying the charge density of the lipid as it passes through the endothelial barrier. The ability of cultured endothelial cells to bind, transport, and degrade low density lipoproteins (LDL) is well documented.212 Further studies indicate that LDL can be modified by endothelial cells so that it becomes more negatively charged, allowing recognition by the modified LDL receptor of the macrophage.221 It may be that endothelial-derived proteoglycans bind to LDL, and in turn, induce such a modification.

It is clear that regions of blood vessels that accumulate proteoglycans have a high propensity to accumulate lipid. For example, studies using the balloon injury model of experimental atherosclerosis indicate that proteoglycans accumulate within regions of injured vessels characterized by endothelial regrowth and not within regions devoid of endothelium.222,223,224 (Figure 9). These studies also indicate that if animals are put on a high-fat diet, lipid accumulates in the re-endothelialized intima, the region characterized by excessive proteoglycan accumulation and not in the de-endothelialized portion225,226 (Figure 9). Such studies raise the interesting possibility that during healing, the regenerating endothelium contributes to the proteoglycan composition of the arterial wall and in some way influences the metabolism of proteoglycans and lipids present in this region of blood vessel.

A number of studies now demonstrate that regions of vessels that have re-endothelialized have higher rates of proteoglycan synthesis and deposition than do de-endothelialized vessels.227,228,229 Co-culture experiments
with endothelial and smooth muscle cells indicate that endothelial cells are capable of stimulating proteoglycan synthesis by smooth muscle cells. These findings suggest that endothelial cells may affect arterial proteoglycan content by: 1) increasing their own proteoglycan synthetic activity or that of adjacent smooth muscle cells, and/or 2) acting as a reverse barrier preventing loss or turnover of proteoglycan. A potential consequence of proteoglycan accumulation in blood vessels is the buildup of lipid (see reference 4 for a review). A number of articles refer to the isolation of PG-LDL complexes from normal and atherosclerotic arteries. Such complexes contain LDL, VLDL, and calcium, and morphologic evidence indicates that these complexes are highly aggregated. Recent work suggests that multiple pools of LDL-PG complexes may be present within blood vessels. For example, complexes extracted with saline contained chondroitin-6-sulfate as the major and hyaluronic acid as the minor glycosaminoglycan and were cholesterol ester-enriched. On the other hand, LDL-PG complexes isolated after collagenase treatment contained mostly hyaluronic acid and minor amounts of chondroitin-6-sulfate. Complexes isolated after elastase contained only hyaluronic acid and were cholesterol ester-poor. These findings suggest that particular LDL-PG complexes may occur within different regions of the extracellular matrix within blood vessels.

Camejo and his colleagues have been studying the interaction of arterial proteoglycan with lipoprotein and have found that arterial CSPG exhibits a marked affinity for LDL and that this interaction may be dependent not only on the type of proteoglycan but also on the surface charge of the LDL. For example, a recent study has shown that a specific sequence enriched in positively charged amino acids in the apo B moiety of LDL effectively binds to proteoglycans. These binding domains are part of the protein that interacts with LDL receptor. Thus, interaction of the proteoglycan with LDL may affect recognition of LDL by its surface receptor. In addition to CSPG, the small arterial DSPG also is capable of forming insoluble complexes with LDL in the presence of calcium, while those proteoglycans containing HS exhibit only minimal lipoprotein binding activities.

Intact proteoglycan is much more effective in precipitating LDL and VLDL than are isolated glycosaminoglycan chains. Removal of the protein core after ß-elimination or protease treatment of the proteoglycan abolishes its insoluble complex-forming ability. The role of net negative charge in complex formation is emphasized by the observation that de-sulfation of the proteoglycan drastically reduces its ability to interact with LDL. Thus, it appears that PG-lipoprotein formation involves a complex series of multiple interactions of protein-protein and protein-carbohydrate. It also is possible that lipids influence the proteoglycan content of the vascular wall and, in turn, influence lipid deposition. Hoff and Wagner demonstrated that after 11 weeks on a hypercholesterolemic diet, porcine aortic glycosaminoglycan concentrations did not differ from that of normocholesterolemic animals, even though lipoproteins accumulated within the aorta. However, the relative amounts of aortic chondroitin sulfate increased, and dermatan sulfate decreased in the hypercholesterolemic animals, indicating that cholesterol feeding may influence the types of proteoglycans that accumulate but not the total quantity. Intense immunostaining using an anti-aortic CSPG antiserum, has been observed in atherosclerotic lesions in the rabbit after lipid feeding (Figure 3). The mechanism responsible for this specific accumulation is unclear, but it may be that lipoproteins influence the synthesis of proteoglycans by the resident cells of the arterial wall. LDL increase the synthesis of proteoglycans by cultured bovine smooth muscle cells, but it is unclear whether specific types of proteoglycans synthesized are affected.

The above studies demonstrate that proteoglycans within the extracellular matrix of blood vessels are capable of binding lipoproteins. However, lipid and lipoprotein concentration...
are found not only within the extracellular matrix, but also within cells of the arterial wall during the genesis of the atherosclerotic lesion. Two cell types that are known to accumulate lipid in developing atherosclerotic lesions are smooth muscle cells and macrophages. Recent studies suggest that proteoglycans may play a role in intracellular lipid accumulation in these cells as well. For example, it is known that macrophages contain few receptors for LDL and, therefore, will not accumulate within macrophages when these cells are incubated with high concentrations of LDL. However, if LDL is modified by acetylation, acetolysis, or malondialdehyde derivation, macrophages dramatically increase their uptake of modified LDL. Enhancement of LDL uptake also occurs when macrophages are incubated with medium containing dextran sulfate. These findings suggest that charge alteration of LDL molecules influences their recognition by macrophages. Thus, overall charge density of lipoproteins could be affected by complexes with negatively charged proteoglycans. Falcone et al. demonstrated that insoluble complexes of LDL, heparin, fibronectin, and collagen were taken up more rapidly in combination and to a greater extent by macrophages than when these cells were incubated with LDL alone. Furthermore, this study demonstrated that catabolism of the endocytosed LDL-complex was greatly diminished, causing cholesterol ester to accumulate within these cells. More recently, Hunt and Camejo demonstrated that incubation of human LDL with human arterial CSPG increased LDL uptake by human monocyte-derived macrophages, indicating that proteoglycan alone is capable of modifying LDL. It is interesting that sulfated polysaccharides such as dextran sulfate inhibit the fusion of phagosomes with lysosomes within macrophages by modifying membrane fluidity of the lysosome. This finding may partially explain why LDL is not degraded and accumulates in macrophages, leading to foam cell formation. However, decreased degradation of LDL may not be the only mechanism by which lipid accumulates in macrophages. Salisbury et al. demonstrated increased cholesterol ester synthesis when macrophages were incubated with aortic proteoglycan and plasma LDL. In addition, Vyas et al. showed that LDL complexed to aortic proteoglycan-hyaluronic acid aggregate was taken up by macrophages, but degraded more rapidly than LDL complexed to proteoglycan monomer. This enhanced degradation also was accompanied by increased cholesterol ester synthesis by the macrophage. This study also showed that acetylated-LDL completely inhibited the degradation of the LDL-PG complex, indicating that the complex may be recognized by the modified LDL receptor of the macrophage.

Atherosclerosis

Studies continue to demonstrate that proteoglycans accumulate within intimal lesions in both large and small vessels involved in atherosclerotic development. These increases appear to involve mainly the CSPG and DSPG families, while some studies indicate little change or even a decrease in HSPG with advancing atherosclerosis (for reviews see references 3, 4, 5, and 250 to 253). Little is known about whether the structural properties of intact proteoglycans are altered in developing atherosclerotic plaques. Wagner et al. noted that CSPG isolated from human fatty-fibrous plaques exhibited less tendency to form aggregates with hyaluronic acid and that the aggregates present in fatty-fibrous arterial plaques were smaller than those present in normal aorta. Altered aggregate formation may have some bearing on arterial calcification (a complication of advanced atherosclerotic plaques) since the degree of proteoglycan aggregation is thought to influence calcification. In addition, the CSPG isolated from human arterial lesions tends to be of larger hydrodynamic size, suggesting either that the synthesis or degradation of this monomer is altered or that it is complexed to other components in arterial lesions. Other minor changes in the structure of arterial proteoglycan have been noted. For example, DSPGs isolated from atherosclerosis-susceptible pigeons contain glycosaminoglycans that are smaller than similar chains isolated from aortic DSPGs of pigeons that are less susceptible to this disease. Whether these differences influence the functional properties of arterial proteoglycans and whether other differences exist must await further investigation.

Conclusion

This review has concentrated on the role that proteoglycans play in vascular wall biology. As summarized in Figure 10, there are a number of ways in which proteoglycans can influence the behavior of vascular cells and, in turn, the normal and pathophysiological properties of blood vessels. Clearly, these macromolecules are of enormous importance in maintaining the viscoelastic state of the vessels, and any change in proteoglycan structure, content, or concentration will severely affect the biophysical properties of the tissue. We now know that a number of factors contribute to alteration of the proteoglycan content of the vascular wall through processes common to both normal development and disease, such as cell proliferation and migration, hormonal stimulation, and multiple ligand interactions. Such "alterations" may, in turn, generate conditions in which different proteoglycans acquire the capacity to "instruct" or to serve as important "informational transducers" regulating key events in vascular wall biology. The future of research in the cell biology of arterial proteoglycans is, indeed, challenging. It represents a critical component in the effort to understand those factors that contribute to cardiovascular disease—the leading cause of death in the United States and Europe.

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Figure 10. A schematic representation of some of the possible roles that proteoglycans play in the biology of the arterial wall.

Functions References
1. Binding of coagulation and anticoagulation factors 19,36,37,38,189,204–208
2. Binding and regulation of enzyme (lipoprotein lipase) activity 215–220
3. Carrier molecule for certain enzyme products and plasma proteins 211,212,214
4. Binding and regulation of growth factor activity 173–184
5. Influencing cell-cell associations 122–124
6. Influencing cell adhesion 104–120
7. Participating in the organization of ECM structures such as basement membranes and regulating permeability 39–43,58–68
8. Influencing endothelial cell migration and proliferation 28,29,126–129,133
9,14,15. Modulation in arterial smooth muscle cell proliferation and migration 77,78,132,134–136,142–147,156–158,169–171
10, 11. Regulation of collagen fibrillogenesis 92–95
12. Maintenance of viscoelastic properties 1,2
13. Modulating calcification 255
16. Influencing intra- and extracellular lipid deposition and turnover 4,232–245

EC=endothelial cells, BM=basement membrane, A III=antithrombin III, T=thrombin, L Pase=lipoprotein lipase, PF4=platelet factor 4, EGF=endothelial-derived growth factor, Lym=lymphocyte, SMC=smooth muscle cell, MAC=macrophage.

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