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Regional Differences in the Distribution of the Proteoglycans Biglycan and Decorin in the Extracellular Matrix of Atherosclerotic and Restenotic Human Coronary Arteries

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Proteoglycans are important constituents of blood vessels and accumulate in various forms of vascular disease. Little is known concerning the proteoglycan composition of restenotic lesions formed after angioplasty and whether the proteoglycan composition of these lesions differs from that of primary atherosclerosis. Accordingly, we sought to characterize the distribution of two proteoglycans, biglycan and decorin, in primary atherosclerotic and restenotic lesions of human coronary arteries. Restenosis (n = 37) and primary (n = 11) lesions obtained from 48 patients by directional atherectomy of human coronary arteries were stained with antibodies against biglycan and decorin. To further characterize the extracellular matrix of restenotic tissues, we studied the co-distribution of these proteoglycans with collagen types I, III, and IV. The loose fibroproliferative tissue seen predominantly in restenosis lesions consistently stained positively for biglycan in patterns of deposition ranging from disseminated to homogeneous. The density and intensity of biglycan staining was correlated with the density of collagen type I and III fiber networks, both of which were observed to interweave among the loose fibroproliferative tissue. The compact connective tissue of primary atherosclerotic plaque was characterized by strong biglycan staining which co-localized with intense collagen type I and III staining. Only basement membrane-like structures rich in collagen type IV demonstrated negative biglycan staining. In contrast, loose fibroproliferative tissue exhibited no significant staining for decorin. Strong immunostaining for decorin, however, was found in primary atherosclerotic plaque. There are thus regional differences in the distribution of extracellular matrix proteoglycans of restenotic and primary human atherosclerotic lesions; these observations suggest that differences established for the biological roles of biglycan and decorin in other organ systems may extend as well to pathologically altered human coronary arteries. (Am J Pathol 1994, 144:962–974)

The production of extracellular matrix (ECM) by smooth muscle cells contributes to the pathogenesis of primary atherosclerosis, as well as lesions which recur after balloon angioplasty, ie, restenosis. Primary atherosclerosis is generally viewed as a chronic process in which lipids and macrophages are considered to play key roles.[1,2] In contrast, experimental studies have suggested that restenosis is dominated by the acute response of vascular smooth muscle cells (VSMC) to arterial injury and is characterized by cell migration,[3] cell proliferation,[4] and associated production of ECM proteoglycans[5–8] and collagen.[9,10] The resulting fibroproliferative tissue (FPT) typical of human restenosis is characterized by foci of hypercellularity in which cells with phenotypic char-

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acteristics of proliferative VSMC are associated with a loose ECM, having tinctorial properties distinct from the ECM of hypocellular, primary atherosclerotic plaque (Figure 1).

Although VSMC proliferation and the resulting hypercellular nature of the FPT have been the focus of most studies of restenosis, it is the ECM which in fact accounts for the bulk volume of these lesions.[11–16] Previous investigations have outlined certain pathways through which deposition of extracellular proteoglycans and collagen are regulated.[17] Moreover, it is now established that these constituents of the ECM may serve as important modulators of cell proliferation, as well as feedback regulators of growth factors governing their synthesis.[18–22] To understand the manner in which proteoglycans contribute to the development of primary and restenotic lesions, and thereby elucidate potential therapeutic implications, it is essential to characterize the individual proteoglycans which comprise these lesions.

Recent studies of both cultured cells and whole tissue have indicated that expression of two proteoglycans in particular, biglycan and decorin, are differentially modulated during ECM elaboration. These two proteoglycans each have a distinct, albeit homologous core protein with a molecular weight of 45 kd and carry either one (decorin) or two (biglycan) chondroitin sulfate or dermatan sulfate side chains, the glycosaminoglycans predominantly found in atherosclerotic tissue.[23] *In vitro* studies performed with cultured cells, including arterial smooth muscle cells, have demonstrated that transforming growth factor-β (TGF-β) upregulates biglycan expression and downregulates decorin expression.[24–27] *In vivo* studies carried out in experimental models of pulmonary and hepatic fibrosis[28,29] have similarly demonstrated TGF-β1 and biglycan are both upregulated during early stages of fibrosis; decorin, in contrast, was found only during the chronic stages of fibrosis. Both biglycan and decorin can bind and neutralize the effect of TGF-β[30]; systemic administration of decorin, in fact, has been shown to inhibit TGF-β-mediated production of extracellular matrix in a rat model of experimental glomerulonephritis.[31] It is therefore possible that proteoglycans such as decorin and biglycan are part of a natural feedback mechanism that regulates the biological activity of TGF-β.[18,30] This may be relevant to neointimal ECM formation, given the documented expression of TGF-β1 in human primary and, to an even greater extent, restenotic lesions.[32–34]

To further characterize the ECM of restenotic tissues, we studied the co-distribution of biglycan and decorin with collagen types I, III, and IV. Expression of collagen types I, III, and IV in cultured cells is also stimulated by TGF-β.[35–37] Decorin has been shown to be strongly bound to collagen type I,[38] but can also inhibit fibrillogenesis of type I and II collagens.[39] Several immunohistochemical[40,41] and biochemical studies[42–44] have suggested that collagen, in particular the interstitial collagen types I and III, represents a major component of fibrotic human atherosclerotic plaque. Collagen IV has been identified as a component of the thick basement membrane layers that surround smooth muscle cells in fibrous atherosclerotic plaque.[41] Little information exists, however, regarding the distribution of these collagen types in relation to the proteoglycans within the ECM of human primary atherosclerotic and restenotic lesions.

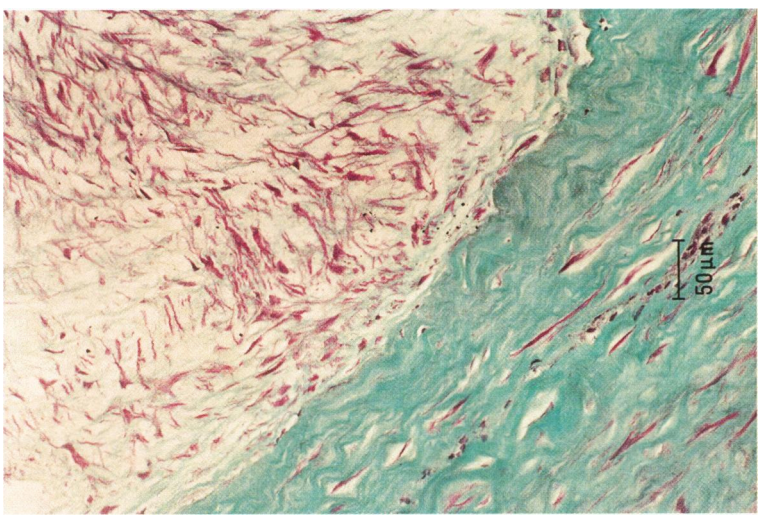

Figure 1. *ET trichrome stain of a typical restenosis lesion. The lesion consists of both FPT with stellate smooth muscle cells embedded in a loose, myxoid extracellular matrix, and hypocellular, dense fibrous plaque (P) (×50).*

Materials and Methods

Patients

Tissue specimens from 48 patients with coronary artery disease were retrieved by directional atherectomy using the Simpson AtheroCath (Devices for Vascular Intervention, Redwood City, CA) as described previously.[45,46] Eleven tissues were obtained from patients with no history of percutaneous revascularization and were therefore classified as primary lesions. The remaining 37 specimens were retrieved from coronary arterial sites previously treated by balloon angioplasty and were therefore classified as restenosis lesions. Thirty-four of these 37 restenosis lesions were retrieved between 1 and 19 months (±SD; 5.1 ± 4.0 months) after previous balloon angioplasty. Three of the 37 restenosis specimens, classified as late restenosis, were obtained 5, 6, and 9 years after balloon angioplasty of the same arterial segment. Control tissues from 4 non- atherosclerotic internal mammary arteries were retrieved during coronary artery bypass surgery.

Tissue Processing

Specimens were fixed immediately after retrieval in ice-cold 4% paraformaldehyde for 2 hours followed by immersion in 30% sucrose/PBS overnight at 4 C. Tissues were embedded in paraffin and cut into 5-μ sections.

Biglycan and Decorin Immunohistochemistry

Antibodies against biglycan (LF 51) and decorin (LF 30) were generous gifts from Dr. L. Fisher (National Institute of Dental Research, Bethesda, MD). The polyclonal antisera, raised against specific synthetic peptides in rabbits, have been described previously and shown to be monospecific.[47–50] In contrast to the biglycan antibody, the staining intensity that could be achieved without significant background staining with the polyclonal decorin antibody was relatively low. Therefore, additional immunostaining for decorin was performed with a specific monoclonal antibody (clone 6-B-6, Seikagaku, Tokyo, Japan) described elsewhere.[51]

After deparaffinization, the sections were treated with 1 U/ml chondroitin ABC lyase (protease-free, from *Proteus vulgaris*; ICN, Covina, CA) for 15 minutes at 37 C to detach glycosaminoglycan side chains from the protein core.[49] Preliminary studies indicated that staining intensity was unaffected by incubation times ranging from 10 to 60 minutes. Omission of the chondroitin ABC lyase digestion step, however, reduced staining intensity, especially with the monoclonal anti-decorin antibody. Endogenous peroxidase activity was blocked with hydrogen peroxide. Nonspecific protein binding of the polyclonal antibodies was blocked by incubation with 20% normal goat serum in PBS. Slides were incubated with primary antibody against biglycan (LF 51) or decorin (LF 30) diluted at 1:200 in PBS, 1% BSA for 2 hours at room temperature. Slides stained with the monoclonal anti-decorin antibody were incubated with diluted normal horse serum before the primary antibody was applied (dilution 1:1000 in PBS, 1% BSA for 1 hour at room temperature). Further immunostaining was then performed using the avidin-biotin-peroxidase technique (Elite Avidin-Biotin Detection System, Signet Laboratories, Dedham, MA). AEC (3-amino-9-ethylcarbazole) was used as a chromogen. Tissues were counterstained with hematoxylin and mounted with aqueous mounting medium under a coverslip. Positive control tissues included human fetal skin,[49] adult skin, and colon tissue.[52] Negative control sections were incubated with normal rabbit serum (1:200 dilution, Sigma Chemical Co., St. Louis, MO) in lieu of primary antiserum, or irrelevant monoclonal antibodies with otherwise similar characteristics to the anti-decorin antibody. To further establish specificity of the polyclonal antibodies, antigen adsorption tests were performed: 1 nmole of the peptide used to raise the antibody (gift from Dr. L. Fisher) was added to 100 μl primary antibody at a dilution of 1:200 in PBS, 1% BSA. After incubation overnight at 4 C, the antigen-antibody solution was centrifuged at 14,000 rpm in a microcentrifuge and the supernatant was used for immunostaining. This procedure did not affect immunostaining when no antigen was added to the antibody solution.

Collagen Immunohistochemistry

Immunostaining for collagen type I was performed with an affinity purified polyclonal rabbit antibody (Biodesign, Kennebunkport, ME),[53] raised against human collagen I. Monoclonal antibodies were used for immunostaining of human collagen type III (clone HWD 1.1, Biogenex, San Ramon, CA)[54] and human collagen type IV (clone CIV 22, Dako, Carpinteria, CA).[55] All antibodies were previously tested in formalin-fixed, paraffin-embedded tissue.

Tissues were predigested with 0.1% trypsin (in 0.1% $CaCl_2$, Tris 0.05 M, pH 7.6 for 7 minutes at 22 C). Further immunostaining was performed using a

biotin-streptavidin-peroxidase kit (StrAviGen Super Sensitive, Biogenex). The antibodies were incubated for 30 minutes at room temperature. All antibodies were diluted in PBS/1% BSA (collagen type I 1:80; collagen type IV 1:100). The collagen type III antibody was prediluted by the manufacturer. Antibody binding was visualized with DAB (diaminobenzidine). Negative controls were incubated with rabbit or mouse immunoglobulins at the same concentration as the primary antibody, or with irrelevant antibodies.

Tissues were also stained with antibodies against smooth muscle actin (HHF 35, Enzo, New York, NY)[56] and macrophages (HAM 56, Enzo)[57] using the avidin-biotin-peroxidase technique (Elite Avidin-Biotin Detection System, Signet Laboratories). Tissues were examined for red-brown (AEC) or brown (DAB) peroxidase reaction product with a bright field Olympus AH2 photomicroscope. Finally, sections of all tissues were stained with an elastic tissue (ET) trichrome stain.

Results

Normal Arteries

Human internal mammary arteries retrieved during bypass surgery disclosed staining for biglycan in all three layers of the vessel wall (not shown). Strong biglycan staining was observed in the intima. This was especially prominent in arteries with some degree of intimal thickening. Streaks of biglycan staining were randomly distributed within the interstitium of the media, either surrounding VSMC or closely associated with elastic laminae. Strong homogeneous staining for biglycan was also seen in the adventitia. A very similar staining pattern was observed with collagen type I and III antibodies.

Staining of normal arteries with polyclonal and monoclonal antibodies against decorin revealed a different pattern, confirming observations from an earlier study of human aorta[51]: intima and media demonstrated no decorin staining, while the connective tissue of the adventitia stained strongly for decorin.

The highest intensity of collagen type IV immunostaining was found immediately underlying the endothelium corresponding to the location of the basal lamina, and at the perimeter of cells in the adventitia. The remaining connective tissue in the adventitia stained negative for collagen type IV. Weak immunostaining was present in the interstitium of the media surrounding VSMC.

Histological Characteristics of Primary and Restenotic Lesions

Specimens retrieved from primary lesions were typically hypocellular and consisted primarily of dense fibrous plaque. The cellular components, confirmed by staining with the muscle-actin-specific antibody HHF35 and the anti-macrophage antibody HAM56, respectively, consisted predominantly of spindle-shaped smooth muscle cells having little cytoplasm and macrophages.

Dense connective tissue typical of primary atherosclerotic plaque was also observed in 35/37 (95%) specimens retrieved from restenosis lesions. In most (23 of 34 [68%]) cases, however, restenosis specimens retrieved within the first 19 months after balloon angioplasty were distinguished from primary lesions by the fact that they included foci of FPT, characterized by stellate smooth muscle cells enveloped by a loose myxoid appearing stroma (Figure 1). Foci of FPT were absent from 3 restenosis specimens retrieved >19 months after angioplasty. Small foci of FPT were found in 2 (18%) of the 11 primary tissues.

ECM Immunostaining of Atherosclerotic Plaque

As indicated above, dense fibrous atherosclerotic plaque was the principal component of atherectomy specimens from primary atherosclerotic and late restenotic lesions and was also present to a varying degree in almost all other restenotic specimens. Typical examples of such fibrous plaque are shown in Figure 2. The ECM of fibrous plaque was characterized by strong staining for biglycan (Figure 2A), which co-localized with staining for collagen types I and III (Figure 2C). Biglycan staining was absent from the sheath-like basement membrane structures surrounding spindle-shaped smooth muscle cells; those biglycan-negative basement membrane sites exhibited strong staining for collagen type IV (Figure 2D). These stained negative for biglycan. Biglycan staining in relation to macrophages was very variable: dense fibrotic plaque infiltrated by some macrophages usually showed intense biglycan staining (Figure 3A). The amorphous material surrounding larger accumulations of macrophages, confirmed by positive staining for the macrophage marker HAM 56 in adjacent sections (Figure 3C), showed very little biglycan staining (Figure 3A).

The distribution of decorin staining in fibrous plaque generally paralleled that of biglycan (Figure 2B); the intensity of staining, however, was more variable than the intensity of the biglycan staining and

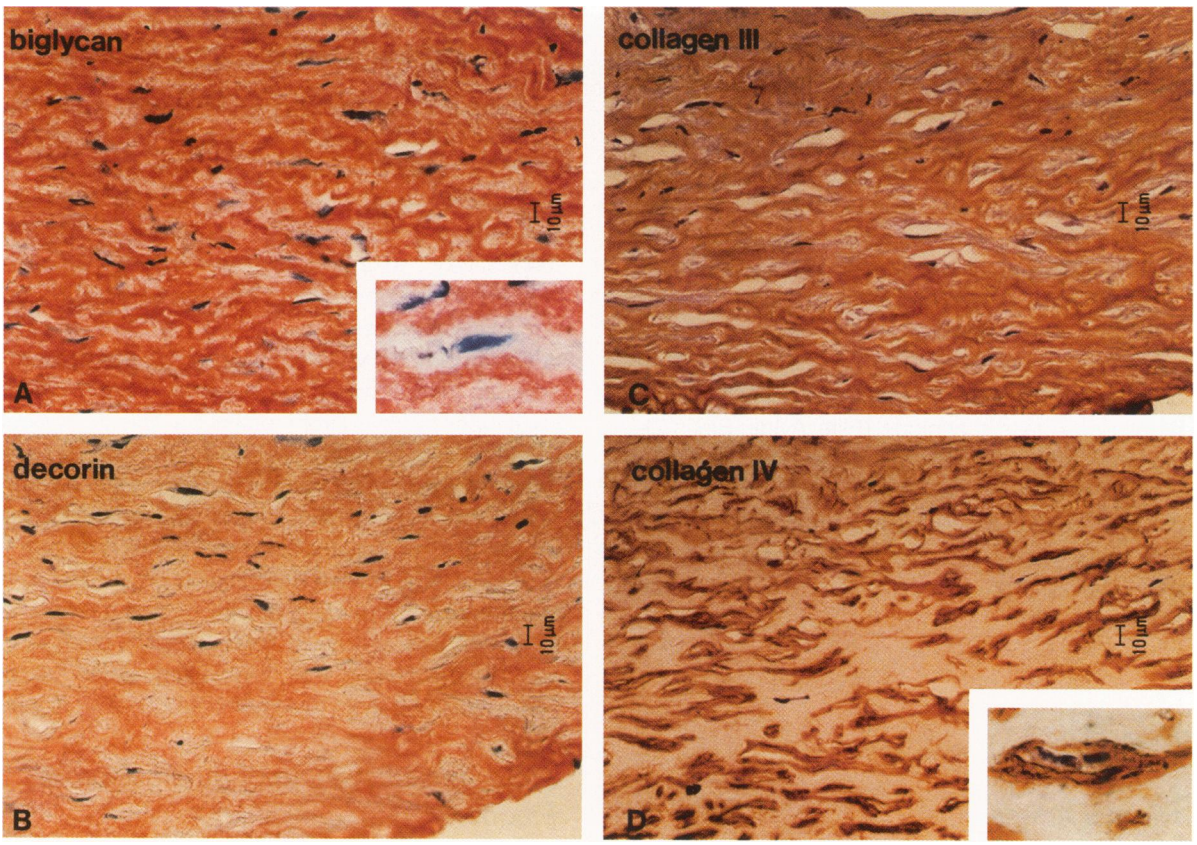

Figure 2. *Primary lesion composed of fibrous atherosclerotic plaque with a moderate cellularity. Serial sections indicate that the extracellular matrix of this lesion is characterized by strong staining for biglycan* (**A**)*, moderate staining for decorin* (**B**)*, and intense collagen III staining* (**C**)*. Collagen type IV* (**D**) *is distributed in a sheath-like manner, enveloping the cells, at a site corresponding to previously described thickened basement membrane*[6,3] *(×100; insets ×250).*

appeared to be related predominantly to the density of the interstitial collagen tissue. In areas with few to absent cells and a very compact matrix, decorin staining could be as intense as biglycan staining (Figure 3B). In areas of primary plaque with numerous macrophages embedded in an amorphous ground substance, decorin staining was weak to absent (Figure 3B).

ECM Immunostaining of Fibroproliferative Tissue

Hypercellular, myxoid zones characteristic for FPT were found predominantly, but not exclusively, in restenosis lesions (see above). FPT foci most often demonstrated a patchy, striated, or speckled pattern of biglycan staining (Figure 4B). The pattern of biglycan staining correlated best with the density of the collagen type I and III network within the FPT. When such foci included extensive numbers of stellate smooth muscle cells surrounded by a loose fibrillar network of collagen types I and III, biglycan deposits were patchy and interwoven with the type I and III collagen fibers (Figure 5, B, D, and E). When collagen type I and III fibers were more dense and compact, biglycan staining was more confluent and intense (Figure 6, B, D, and E). An inverse relationship between staining for biglycan and collagen type IV was observed within foci of FPT, as was the case for primary atherosclerotic lesions. The perimeter of smooth muscle cells was typically enveloped by collagen type IV (Figures 5F and 6F); staining for biglycan, as well as decorin, was excluded from these type IV collagen-rich envelopes. Frequently, staining of collagen type IV was also observed in the extracellular space, not associated with cells (Figure 5F), presumably representing thick fibrous or membrane-like fragments.

Decorin was virtually absent from the loose ECM of foci of FPT characterized by patchy biglycan staining (Figures 4C and 5C). Decorin staining of the FPT-ECM was limited to weak staining intensity in areas with a

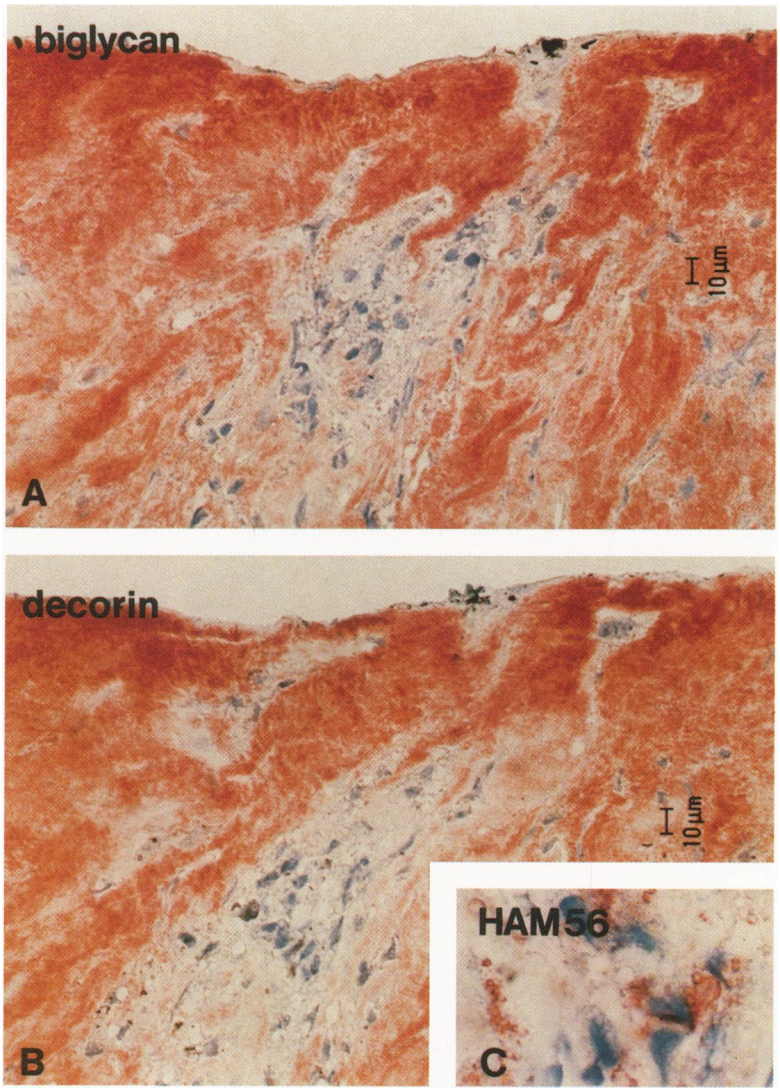

Figure 3. *Extracellular matrix in serial sections of a primary lesion which is notably hypocellular, except for a focal collection of macrophages. Intense staining of very similar distribution is found for biglycan* (**A**) *and decorin* (**B**) *in the hypo- to acellular portions of the plaque. The cells in the center of the figure stain positively for the macrophage marker HAM56* (**C**). *The amorphous material directly surrounding these macrophages shows no significant staining for either biglycan or decorin* (×*100*; C, ×*250*).

denser collagen matrix and more confluent biglycan staining (Figures 4 and 6), often in the border zone between FPT and fibrous plaque.

Discussion

In this study we have used immunohistochemical methods to describe the composition of the ECM in human primary and restenotic coronary arteries with a focus on the proteoglycans biglycan and decorin and the relationship of these proteoglycans to deposits of collagen types I, III, and IV. We specifically sought to characterize the loosely woven, myxoid-appearing ECM that surrounds foci of stellate smooth muscle cells. Earlier autopsy studies suggested that this so-called FPT is characteristic of intimal hyperplasia in restenosis lesions after revascularization.[11,13] Previous study of tissue specimens retrieved by directional atherectomy[12,15] indicated that FPT occurs more commonly in restenotic than in primary atherosclerotic lesions. While experiments performed in animals have demonstrated that intimal hyperplasia resulting from balloon injury incorporates proteoglycans[5–8] and collagen,[9,10] little is known

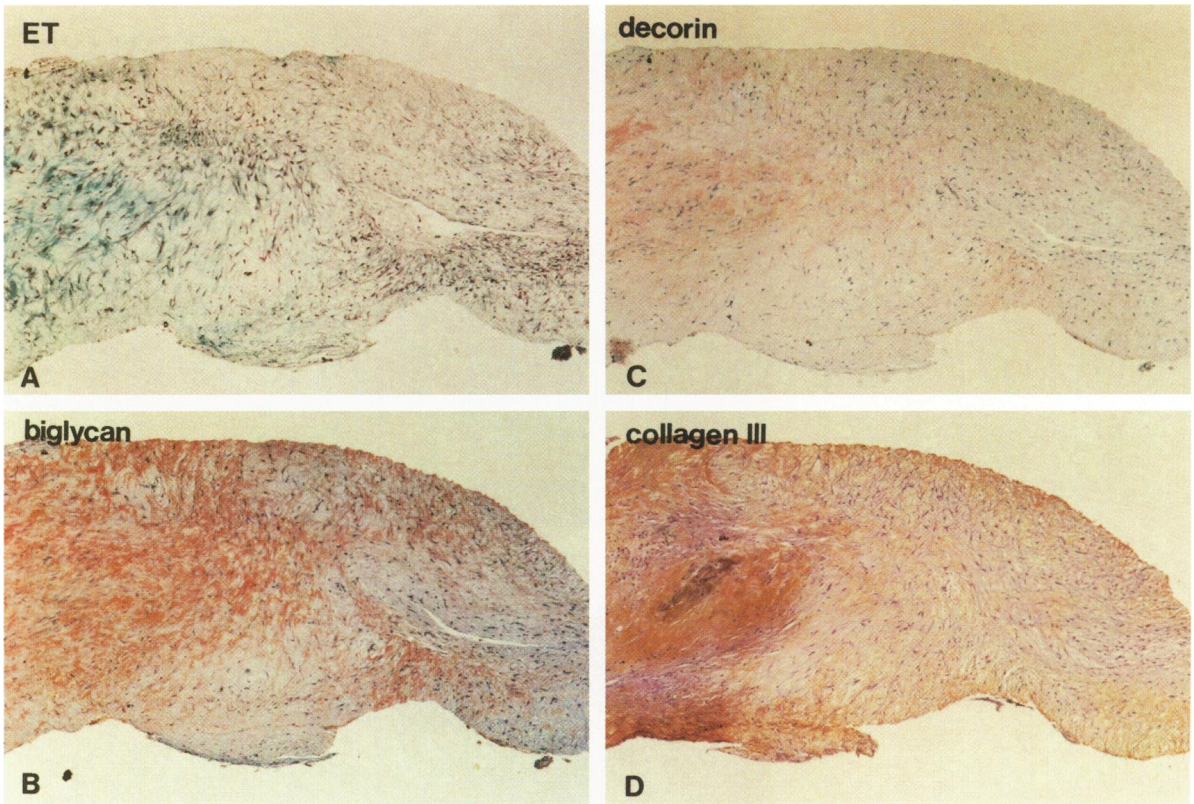

Figure 4. *FPT from a restenotic lesion retrieved 2.5 months after angioplasty. ET trichrome stain* (**A**). *Biglycan is present throughout the tissue* (**B**) *with the exception of a few cell layers* (arrow). *Weak decorin staining* (**C**) *is restricted to an area in which serial sections also show intense staining for biglycan and collagen type III* (**D**) (×*20*).

regarding the distribution of proteoglycan and collagen subtypes in human tissues. Furthermore, comparative analysis of proteoglycan and collagen constituents of human primary atherosclerotic lesions *versus* recurrent (restenosis) lesions has not been previously undertaken.

Staining of normal internal mammary arteries confirmed earlier biochemical and immunohistochemical studies of human aorta,[51,58] which established that the normal arterial intima and media are rich in biglycan, whereas decorin is absent from the normal vessel wall with the exception of the adventitia.

The observations described in the current study indicate that extracellular deposits of biglycan are characteristic of foci of FPT. Examination of serial sections revealed that biglycan staining was intimately related to the density and intensity of collagen type I and III staining in the same area. Collagen type I and III deposits were typically observed as a network of randomly arranged fibers; the density of the fiber network, however, was variable and ranged from very loose to dense. Decorin immunostaining was absent from FPT foci in which biglycan staining was patchy and associated with a loose collagen network. Staining for decorin was limited to weak staining intensity in the more compact transition zone between loose FPT-ECM and the dense connective tissue typical of advanced lesions composed of hypocellular fibrous plaque. These latter areas typically also showed strong staining for biglycan. These findings *in toto* suggest that accumulation of collagen and biglycan, followed at later stages by decorin, represent key constitutional features of the matrix remodeling that occurs after angioplasty. Foci of FPT were, in addition, characterized by intense staining for collagen type IV, corresponding to the basement membrane at the perimeter of VSMC. Structures rich in collagen type IV, such as cell surfaces, typically demonstrated weak staining for biglycan.

Hypocellular atherosclerotic plaque composed of dense connective tissue was seen in all primary lesions but was also observed in 95% of all restenosis lesions. Intense staining for both biglycan and decorin was a consistent feature of such tissue and co-localized with staining for collagen types I and III. The intensity of decorin staining was particularly

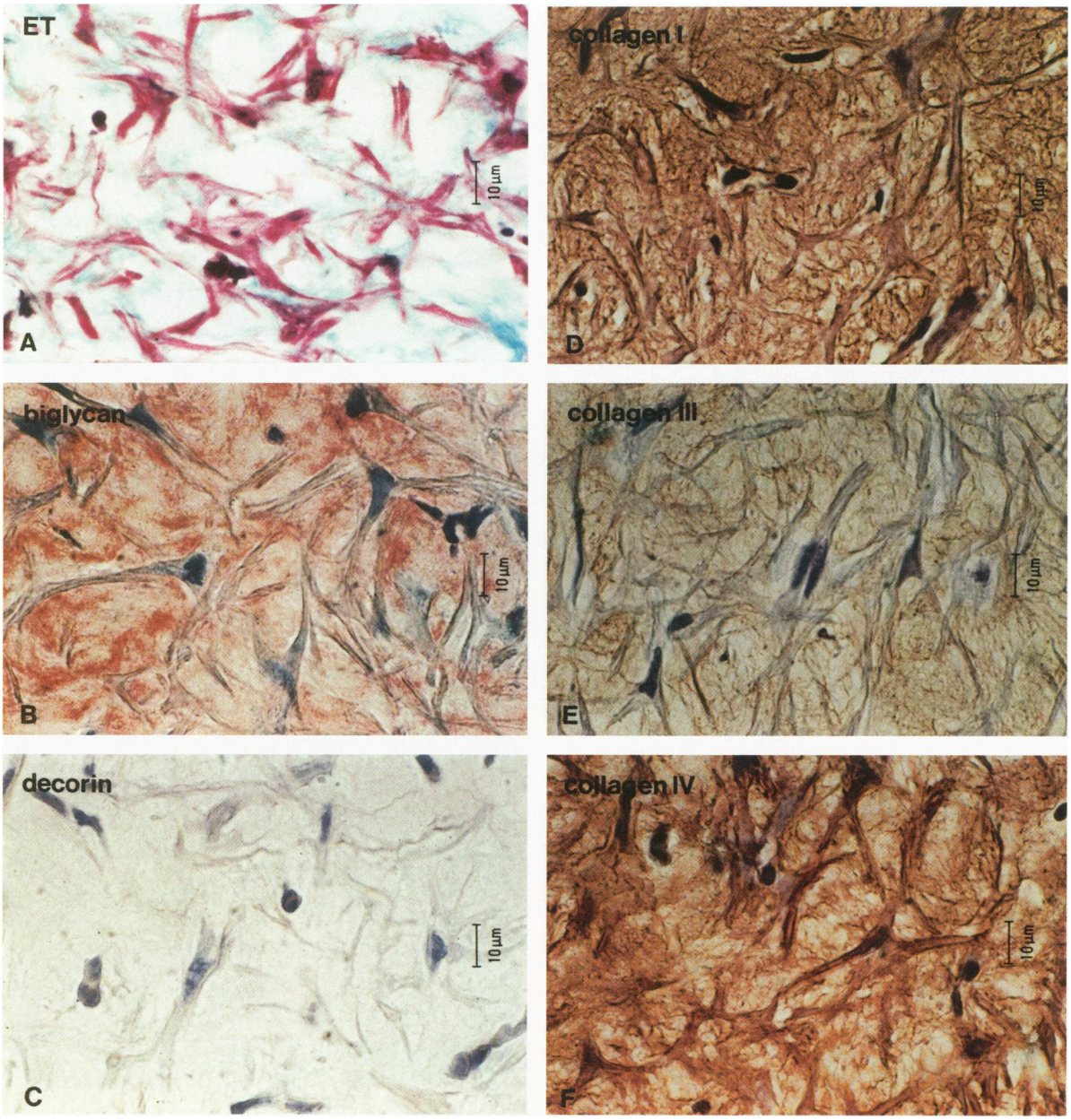

Figure 5. *High-magnification photomicrographs taken from serial sections of a restenosis lesion (6 months after angioplasty) showing a focus of fibroproliferative tissue. Stellate-shaped smooth muscle cells (HHF35 positive, not shown) are embedded in a loose ECM (ET trichrome stain,* **A**). *Immunostaining shows disseminated extracellular biglycan deposits* (**B**), *no decorin* (**C**), *a loose network of collagen type I* (**D**) *and type III* (**E**). *Staining for collagen type IV* (**F**), *while also present in an interstitial distribution, is most intense around the perimeter of cells* (×250).

strong in areas in which the matrix was most compact. Only the pericellular sites with intense staining for collagen type IV showed no staining for biglycan and decorin, indicating that these proteoglycans are not constituents of the thick layers of basement membranes which are present in primary atherosclerotic plaque. The staining pattern we have described for the collagen types I, III, and IV in hypocellular areas of compact extracellular matrix is consistent with earlier descriptions of primary atherosclerotic plaque.[41,53]

We[32,33] and others[34] have recently shown that expression of TGF-β is increased in restenotic lesions retrieved from human coronary and peripheral arteries. The neointima of rat carotid arteries subjected to balloon injury demonstrates similarly increased

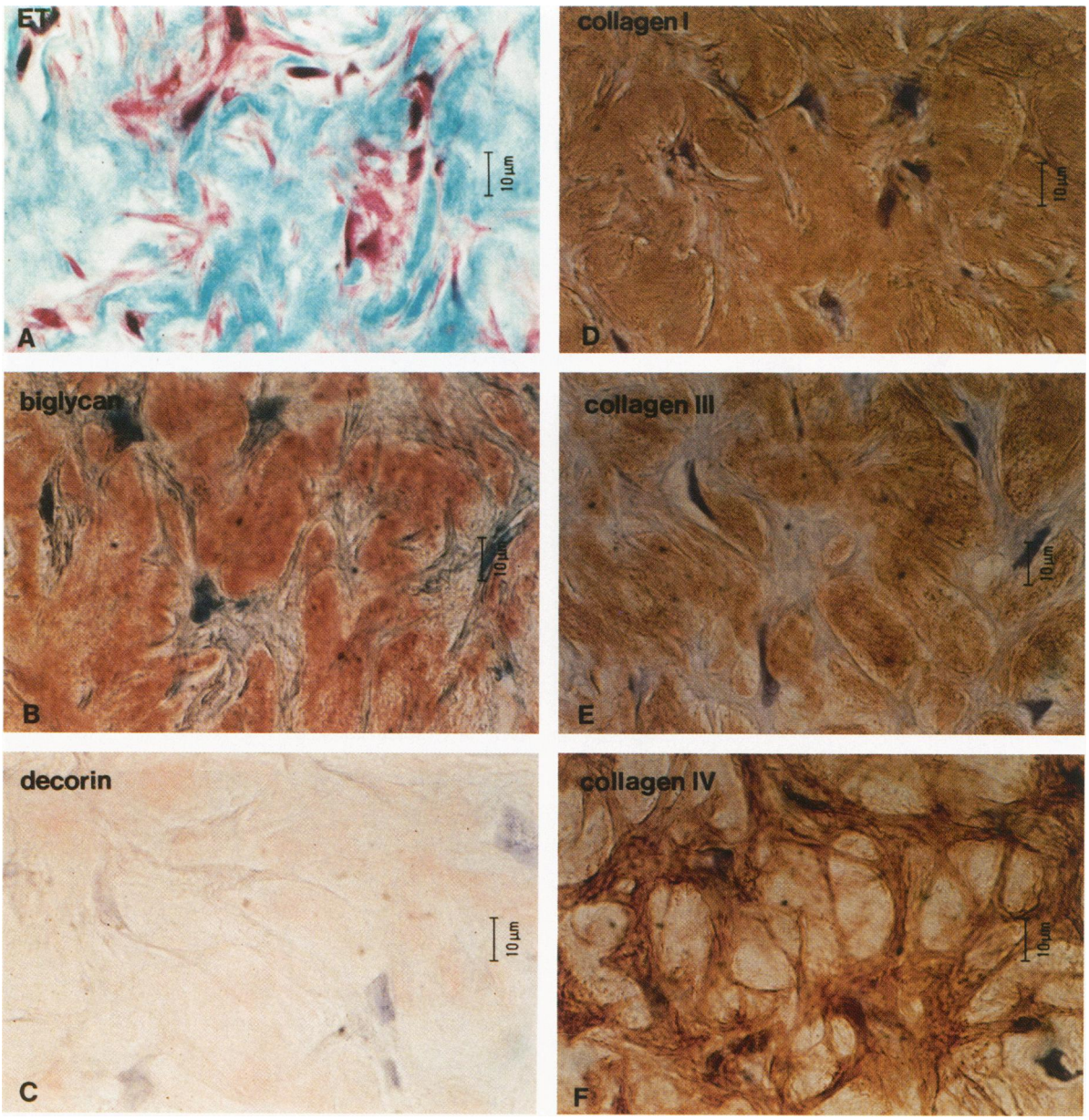

Figure 6. *Serial sections from a different area of the same specimen shown in Figure 5. Stellate smooth muscle cells in this area are embedded in a more compact ECM. ET trichrome stain* (**A**). *Extracellular biglycan staining* (**B**) *is confluent and intense, in contrast to very weak decorin staining* (**C**). *Immunostaining for collagen type I* (**D**) *and type III* (**E**) *reveals a very dense array of collagen fibers. Collagen type IV staining is restricted to the cell perimeter* (**F**) (×250).

TGF-β1 expression.[59] Previous work has shown that when TGF-β1 is used to stimulate cultures of human fibroblasts or primate VSMC, biglycan expression is upregulated, whereas decorin expression is unaffected or even reduced.[24,26] TGF-β also increases production of collagen types I and III in cultured vascular smooth muscle cells.[35,36] Coordinated upregulation of TGF-β1, biglycan, and collagen III mRNA levels has also been observed in a rat model of bleomycin-induced pulmonary fibrosis,[28] reaching a maximum at day 10 after bleomycin instillation; decorin mRNA was observed at the same time point to be reduced and then increased at later time points. Similar findings were reported for of TGF-β1, biglycan, and decorin expression in experimentally induced liver fibrosis in the rat[29]: 2 to 4 weeks after initiation of thioacetamide administration, expression of biglycan and TGF-β1 increased markedly. Decorin expression was detectable only at 2 months, ie, after transition to the chronic fibrotic stage. It is thus pos-

sible that increased expression of TGF-β is responsible for the differential regulation of biglycan and decorin production observed in FPT.

The observation that weak decorin staining was found in certain areas of FPT with a dense array of collagen fibers and, to a much greater extent, in fibrotic atherosclerotic plaque is intriguing, given that decorin was not observed in normal arterial intima and media. Recent studies have suggested that decorin, and possibly also biglycan, are candidate components of a feedback system which regulates the biological activity of TGF-β. Yamaguchi et al[60] have shown that high-level decorin expression in Chinese hamster ovary cells has a dramatic effect on their morphology and growth properties. This effect appears to be due in part to the ability of the decorin core protein to bind TGF-β.[30] Likewise, when Border et al[31] administered decorin intravenously to rats with experimental glomerulonephritis, reduced deposits of fibronectin and tenascin were attributed to the TGF-β inhibiting properties of decorin. While it is known that biglycan can also bind TGF-β,[30] it has not been previously determined whether biglycan, administered exogenously or produced endogenously, results in biological effects similar to those seen with decorin.

The differential expression of biglycan and decorin observed in this study is also consistent with proteoglycan expression observed in developing human tissues: while biglycan has been found principally in association with tissues involved in active morphogenesis and differentiation, decorin has been associated with type I and type II collagen-rich connective tissues such as dermis and tendons.[49]

Unlike biglycan, decorin is strongly bound to collagen type I[38] and has been previously shown to inhibit fibrillogenesis of type I and II collagens.[39] It is not known whether a functional relation between biglycan and other collagen types exists. It has therefore been suggested that TGF-β-induced reduction in decorin production might secondarily result in enhanced fibrillogenesis of newly synthesized type I collagen molecules during tissue repair.[24] The differing structure of this collagen tissue, perhaps represented in our study by the loose collagen network found in foci of FPT, may be necessary for the migration and penetration of smooth muscle cells in this remodeling tissue. Factors responsible for decorin deposits associated with foci of dense fibrotic tissue in human vascular lesions remain enigmatic. *In vitro* studies of arterial smooth muscle cells[61] and skin fibroblasts[62] have demonstrated that decorin expression may be stimulated by interleukin-1. Infiltration with macrophages, a source of interleukins, was in our and other studies[57] a common feature of primary atherosclerotic plaque, but has not proved to be a characteristic feature of FPT. Whether the interaction of macrophages and smooth muscle cells contributes to the accumulation of decorin in primary atherosclerotic plaque requires further study.

These observations thus document regional differences in the distribution of ECM proteoglycans among primary atherosclerotic and restenotic human lesions. Decorin may well represent a characteristic feature of mature connective tissue with little residual proliferative activity; biglycan, in contrast, may be more directly involved in the remodeling of proliferative tissue. Further experimental studies will be required to elucidate the precise biological roles of biglycan and decorin in the development of atherosclerosis and restenosis to better define potential therapeutic implications.

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