Decorin Modulates Fibrin Assembly and Structure*

Tracey A. Dugan, Vivian W.-C. Yang, David J. McQuillan, and Magnus Höök

From the 1Center for Extracellular Matrix Biology, Institute of Biosciences and Technology, Texas A&M Health Science Center, Houston, Texas 77030, the 2Graduate Institute of Biomedical Materials, Taipei Medical University, Taipei 110, Taiwan, and the 3LifeCell Corporation, Branchburg, New Jersey 08876

Emerging evidence indicates that fibrin clotting is regulated by different external factors. We demonstrated recently that decorin, a regulator of collagen fibrillogenesis and transforming growth factor-β activity, binds to the D regions of fibrinogen (Dugan, T.A., Yang, V. W.-C., McQuillan, D.J., and Höök, M. (2003) J. Biol. Chem. 278, 13655–13662). We now report that the decorin-fibrinogen interaction alters the assembly, structure, and clearance of fibrin fibers. Relative to fibrinogen, substoichiometric amounts of decorin core protein modulated clotting, whereas an excess of an active decorin peptide was necessary for similar activity. These concentration-dependent effects suggest that decorin bound to the D regions sterically modulates fibrin assembly. Scanning electron microscopy images of fibrin clotted in the presence of increasing concentrations of decorin core protein showed progressively decreasing fiber diameter. The sequestration of Zn2+ ions from the N-terminal fibrinogen-binding region abrogated decorin incorporation into the fibrin network. Compared with linear thicker fibrin fibers, the curving thin fibers formed with decorin underwent accelerated tissue-type plasminogen activator-dependent fibrinolysis. Collectively, these data demonstrate that decorin can regulate fibrin organization and reveal a novel mechanism by which extracellular matrix components can participate in hemostasis, thrombosis, and wound repair.

Fibrinogen and fibrin participate in an array of interactions contributing to hemostasis (1). These interactions follow tissue trauma triggering platelet activation and the coagulation cascade. Thrombin converts fibrinogen into fibrin by releasing the α- and β-chain fibrinopeptides (2, 3). Fibrin then rapidly assembles into prototypils with a half-molecule, staggered, overlapping, and end-to-end arrangement (4–6). Prototypils coalesce into fibers through sites of lateral interaction that have not yet been completely characterized (7). Two types of branches that contribute differently to the physical properties of the fibrin network may form (8). Thrombin also generates factor XIIIa, which cross-links self-associating fibrin, thus yielding a covalently stabilized provisional matrix (9, 10).

The extracellular matrix plays important roles in wound repair and vascular disease. Beyond a key role in hemostasis, the fibrin-rich clot containing fibronectin serves as a provisional matrix supporting the migration and proliferation of inflammatory cells and fibroblasts (11, 12). Wound strength improves as matrix remodeling replaces these temporary structures with collagen fibers.

Decorin is expressed as an abundant small leucine-rich proteoglycan by a variety of cells, including cultured vascular smooth muscle cells, pericytes, and sprouting endothelial cells (13–15). Immunohistochemical staining has revealed decorin in the subendothelial matrix of healthy and diseased vessels (16, 17). Several independent studies have detected decorin in mouse and human atheromas as well as in the macrophage-replete lipid core and the collagenous fibrous cap (18–24). Moreover, decorin was detected in the mineralized region of vascular lesions following sudden death (25). In contrast to the proteoglycans of normal blood vessels, the dermatan sulfate content of decorin is decreased during atherosclerosis (26). This observation could explain the reduced ability of plaque-derived decorin to accelerate the inactivation of thrombin by heparin cofactor II. In vitro, the antithrombin activity of heparin cofactor II is potentiated by dermatan sulfate-enriched decorin or purified galactosaminoglycans, but not by chondroitin sulfate glycoforms or the isolated decorin core protein (27).

The small leucine-rich proteoglycans compose a family of 11 extracellular matrix glycoproteins with sequence and structural similarity (28, 29). A shared phenotype among small leucine-rich proteoglycan null mouse strains is a tissue-restricted defect in collagen fiber assembly (30–33). Decorin-deficient mice present with fragile skin reminiscent of dermatosparaxis (30). Ultrastructural images of the dermal collagen from these mice uncovered abnormal variability in fiber girth and interfibrillar spacing. Mechanistic studies in vitro have demonstrated that the core protein of decorin mediates collagen binding requisite for regulating fibrillogenesis (34–38).

Structural models of decorin core protein have revealed a non-globular fold with ample surface for an array of interactions (39, 40). The central domain consists of 12 tandem leucine-rich repeat motifs that potentially can offer binding sites for collagens I, V, and VI; fibronectin; the epidermal growth factor receptor; and transforming growth factor-β (27, 36–38, 41–45). N- and C-terminal segments containing...
pairs of cysteinyl residues flank the leucine-rich repeat domains of decorin, all other small leucine-rich proteoglycans, and many proteins of the leucine-rich repeat superfamily (28, 29, 46). These disulfide bonds are believed to stabilize the overall modular structure of leucine-rich repeat proteins (35, 40). Our previous study showed that the N-terminal regions of the decorin and biglycan core proteins bind Zn\(^{2+}\) ions when present at low micromolar concentrations (47). This corroborates analytical ultracentrifugation results showing that biglycan proteoglycan occurs as a hexamer in the presence of Zn\(^{2+}\) ions while in monomer-dimer equilibrium with EDTA (48). Subsequently, we discovered that Zn\(^{2+}\) ions enable the N-terminal region of decorin core protein to self-associate and specifically recognize the D regions of fibrinogen (49).

This study was designed to assess the effects of decorin-fibrinogen interaction on fibrin clot assembly and structure. We show here that decorin core protein or an N-terminal decorin peptide mimetic containing the fibrinogen-binding site can modulate fibrin clotting. In this process, decorin becomes incorporated into the fibrin network. Fibrin clots formed in the presence of decorin and observed by scanning electron microscopy (SEM) exhibited a novel structure and underwent accelerated fibrinolysis when incubated with plasminogen plus tissue-type plasminogen activator, but not with pre-activated plasmin. Thus, we provide evidence that decorin can act as a novel regulator of fibrin(ogen) function during hemostasis, thrombosis, and wound repair.

**EXPERIMENTAL PROCEDURES**

**Expression and Purification of Recombinant Decorin Core Proteins**—Recombinant human decorin core protein was produced using a vaccinia virus-based mammalian cell culture system as described previously (49, 50). To prevent decorin core protein from being generated with galactosaminoglycan polysaccharides, we used CHO-745 cells deficient in xylosyltransferase activity (51). Next, decorin core protein bearing an N-terminal His tag was purified from the cell culture medium on an Ni\(^{2+}\)-chelated chromatographic matrix (49, 50). The purity and identity of decorin core protein were verified by SDS-PAGE with Coomassie Brilliant Blue R-250 staining and by Western blotting with rabbit anti-decorin polyclonal antiserum PR2 and alkaline phosphatase-conjugated goat anti-rabbit IgG (Bio-Rad) (50). This analysis revealed a doublet of bands that was shown previously to represent intact decorin core proteins harboring two or three N-linked oligosaccharides. Diffusely staining decorin proteoglycans were not observed. Decorin concentrations were quantified using a calculated extinction coefficient at 280 nm of 19,820 M\(^{-1}\) cm\(^{-1}\). To generate core protein depleted of metal ions, 10 \(\mu M\) decorin was mixed with 1 mM EDTA and incubated overnight. Western blotting was employed to confirm that this incubation did not result in the proteolytic fragmentation of decorin.

A recombinant peptide mimetic of the murine decorin N-terminal domain was expressed in *Escherichia coli*, purified, and refolded with Zn\(^{2+}\) ions as described previously (47, 49). The decorin N-terminal domain comprises a 4-residue vector-derived sequence, followed by 41 residues corresponding to the N-terminal segment of mature mouse decorin core protein. Peptide concentrations were calculated with an extinction coefficient at 280 nm of 3280 M\(^{-1}\) cm\(^{-1}\).

**Clotting Assay**—Human fibrinogen depleted of plasminogen, von Willebrand factor, and fibronectin (FIB 3, Enzyme Research Laboratories) was incubated for 1.5 h at ambient temperature in the absence or presence of decorin core protein or peptide over a range of concentrations and under the solution conditions specified in each figure legend. Aliquots of 90 \(\mu l\) from each mixture containing 2.0 \(\mu M\) fibrinogen were dispensed into separate wells of a flat-bottomed microtiter plate (Immulon, Dynatech Laboratories, Inc.) (52). A total of four to six clotting reactions were simultaneously initiated by the addition of 10 \(\mu l\) of thrombin (Novagen) to each well through a multichannel pipette. The final thrombin concentration was 0.25 units/ml. Fibrin clotting was traced by measuring absorbance values at 405 nm over time at 30-s intervals using a Thermomax microplate reader (Molecular Devices Corp.) online with Softmax software (Phoenix Technologies Ltd.). The data were imported into KaleidaGraph (Synergy Software) for analysis and graphical presentation in PowerPoint (Microsoft). The slope of the steepest segment of each clotting curve was calculated and multiplied by 1000 to give the rate in milli-absorbance/min. Clot turbidity measured 10 min after thrombin addition was reported as the final absorbance at 405 nm because it was found to be equivalent to the turbidity observed 16 h later.

**Scanning Electron Microscopy**—Fibrin clots were prepared in the absence or presence of decorin in duplicate at the concentrations and conditions specified in the figure legends. Each clot was formed on Parafilm within a circular ring cut from the cap of a 200-\(\mu l\) PCR tube (Contiental Lab Products) (52). Thrombin in a 5-\(\mu l\) drop was initially placed in the center of the ring and mixed with 45 \(\mu l\) of a solution containing fibrinogen. After 3 h, the rings with adhering clots were gently removed from the Parafilm and placed in the wells of a microtiter plate containing 150 \(\mu l\) of 50 mM sodium cacodylate buffer (pH 7.4). For buffer exchange, clots were incubated three times in 150 \(\mu l\) of cacodylate buffer for 10 min. The clots were next fixed with 2% (v/v) glutaraldehyde (Sigma) in cacodylate, rinsed three times, stained with freshly opened osmium tetroxide (Sigma), and dehydrated through a series of ethanolic solutions. The dehydrated clots were transported in wells containing 100% ethanol, followed by critical point drying in a Balzers apparatus and sputter coating with gold/palladium particles ~20 nm in diameter. Images were acquired with a Zeiss 1530VP field emission scanning electron microscope (Carl Zeiss MicroImaging, Inc., Thornwood, NY) at the Texas A&M University Microscopy and Imaging Center. A 5.0-mm working distance and a 1.0-kV accelerating voltage were employed. For each set of conditions, at least three separate experiments were conducted in which two clots were observed. From different

---

3 The abbreviations used are: SEM, scanning electron microscopy; HBS, Heps-buffered saline; t-PA, tissue-type plasminogen activator; CHAPS, 3-(3-cholamidopropyl)dimethylammonio)-1-propanesulfonic acid.
Decorin Modulates Fibrin

**FIGURE 1.** Thrombin-induced fibrin clotting in the presence of increasing concentrations of decorin core protein or peptide. A, fibrinogen was diluted to a concentration of 2.0 μM in 20 mM Hepes, 150 mM NaCl, 0.1% (w/v) CHAPS, 5 mM ε-aminocaproic acid, 5 mM CaCl2, and 20 μM ZnCl2 (pH 7.4) alone (○) or with decorin core protein at a concentration of 0.2 μM (△), 0.4 μM (■), 1.0 μM (□), or 2.0 μM (●). B, fibrinogen was diluted to a concentration of 2.0 μM in 20 mM Hepes, 150 mM NaCl, 5 mM ε-aminocaproic acid, 5 mM CaCl2, and 20 μM ZnCl2 (pH 7.4) alone (○) or with a recombinant peptide mimetic of the decorin core protein N-terminal domain (DcnNTD) at a concentration of 2.0 μM (△), 6.0 μM (■), 12 μM (□), or 30 μM (●). In A and B, the clotting reactions proceeded at −25 °C following the addition of 0.25 units/ml thrombin. The turbidity of clotting solutions was monitored simultaneously using a plate reading spectrophotometer. The mean ± S.D. of four polymerization curves was calculated for each set of conditions. The results from a quantitative analysis are given in Tables 1 and 2.

regions of each image, 50 fibers were randomly selected and measured using NIH Image Version 1.61 according to calibration instructions with the software. Images were imported into PowerPoint, and any adjustment in size was performed carefully to prevent distortions.

**Immunodetection of Decorin in Fibrin Clot Extracts**—Fibrin clots were prepared as described above for the fibrin clotting assay in the absence or presence of decorin core protein with divalent cations (Zn2+ and Ca2+) or with EDTA. Two clots formed under identical conditions were removed from the microtiter plate wells, combined, and sedimented by centrifugation. The supernatant containing soluble unincorporated proteins was removed from the pelleted fibrin clot matrix. The clots were resuspended in 60 μl of Hepes-buffered saline (HBS; pH 7.4) plus 20 μl of 4× reducing sample loading buffer (50 mM Tris (pH 6.8), 2% (w/v) SDS, 10% (v/v) glycerol, 0.001% (w/v) bromphenol blue, and 4% (v/v) 2-mercaptoethanol) and solubilized by boiling for 10 min. The clot extracts were then snap-frozen in a dry ice/ethanol bath and stored at −20 °C prior to analysis by Western blotting.

The clot extracts were fractionated by SDS-PAGE through 4% stacking and 10% separation slab gels in accord with standard procedures. Following pre-activation of an Immobilon-P membrane (Millipore Corp.) by rinsing with methanol, proteins were transferred to the membrane in a semidry transfer cell (Bio-Rad). The membrane was incubated overnight at 4 °C in 10% (w/v) nonfat dry milk reconstituted in HBS to saturate the remaining protein-binding sites on the membrane. Subsequently, the membrane was washed twice with HBS containing 0.5% (v/v) Tween 20 (HBST). A solution comprising PR2 antiserum diluted 2000-fold in HBS with 0.1% (w/v) nonfat dry milk was incubated with the membrane for 30 min. Following two washes with HBST, alkaline phosphatase-conjugated goat anti-rabbit IgG diluted 5000-fold in HBS with 0.1% (w/v) nonfat dry milk was mixed with the membrane for 20 min. An alkaline phos-

Turbidity measurements were converted to percentages of clot lysis using Equation 1,

\[
\% \text{ clot lysis} = 100\% \times \left( \frac{A_{\text{initial}} - A}{A_{\text{initial}}} \right)
\]

where \(A_{\text{initial}}\) and \(A\) represent the clot turbidity before initiating lysis and at each time recorded, respectively. The analysis and graphical display were performed as described above under “Clotting Assay.”

**RESULTS**

**Effects of Decorin on Fibrin Clotting**—The N-terminal region of decorin core protein specifically interacts with the D regions of fibrinogen (49). Following thrombin conversion of fibrinogen into fibrin, the D regions play essential roles in the assembly of protofibrils and fibers (1). Consequently, we explored whether decorin binding to the D regions alters fibrin assembly. A previous study has shown that dermatan sulfate-bearing decorin proteoglycans can inhibit thrombin through the activation of heparin cofactor II (27). Thus, to observe an impact of decorin-fibrinogen interaction on clotting, our experiments were conducted with a recombinant galactosaminoglycan-free form of decorin core protein (49–51).

Initially, fibrinogen was incubated alone or with increasing concentrations of decorin core protein. Aliquots of these solutions in microtiter plate wells were then simultaneously clotted by adding thrombin (Fig. 1A). The subsequent rising turbidity has been shown to indicate the progress of fibrin clot development. Moreover, the turbidity of the clot is proportional to the average fiber girth. A quantitative analysis of the clotting curves revealed the extent to which both the assembly rate and final absorbance of fibrin clots decreased with increasing concentrations of decorin core protein (Table 1). Relative to the clotting of 2.0 μM fibrinogen in the absence of decorin, the addition of a substoichiometric concentration of 0.2 μM decorin reduced the rate and final absorbance by 31 and 22%, respectively. With

phosphatase-induced stain developed as the membrane was incubated with 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium (Bio-Rad). Two bands corresponding to decorin core proteins appeared within 10 min at ambient temperature. Similar results were obtained in repeated experiments. The blots were scanned and imported into PowerPoint.

**Fibrinolysis Assay**—Fibrin clots formed in the presence of divalent cations as described above under “Clotting Assay,” but with the omission of the plasmin inhibitor ε-aminocaproic acid, were incubated with 100 μl of HBS containing either tissue-type plasminogen activator (t-PA) with Glu-plasminogen or plasmin (53). The decreasing turbidity at 405 nm was traced for 6 h.

phosphatase-induced stain developed as the membrane was incubated with 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium (Bio-Rad). Two bands corresponding to decorin core proteins appeared within 10 min at ambient temperature. Similar results were obtained in repeated experiments. The blots were scanned and imported into PowerPoint.

**Fibrinolysis Assay**—Fibrin clots formed in the presence of divalent cations as described above under “Clotting Assay,” but with the omission of the plasmin inhibitor ε-aminocaproic acid, were incubated with 100 μl of HBS containing either tissue-type plasminogen activator (t-PA) with Glu-plasminogen or plasmin (53). The decreasing turbidity at 405 nm was traced for 6 h.

- **Fibrin Modulates Fibrin**
- **Immunodetection of Decorin in Fibrin Clot Extracts**
- **RESULTS**
- **Effects of Decorin on Fibrin Clotting**
- **Fibrinolysis Assay**
increasing decorin concentrations up to 1.0 μM, fibrin clotting was further attenuated. However, clots developed similarly whether decorin was present at a concentration of 1.0 or 2.0 μM, indicating saturation. Thus, both the rate of fibrin clot formation and the structure of clots formed are affected by the presence of decorin core protein. Furthermore, the maximum effect is seen with a substoichiometric concentration of decorin.

Our previous study showed that a recombinant peptide mimetic of the N-terminal region of decorin core protein completely inhibits the binding of intact decorin proteoglycan to fibrinogen (49). Thus, we tested the ability of the decorin N-terminal domain to alter thrombin-induced clotting (Fig. 1B). Indeed, decorin peptide also altered fibrin clotting, yet much less potently compared with the intact core protein. The clotting of 2.0 μM fibrinogen was increasingly attenuated by the core protein at concentrations ranging from 0.2 to 1.0 μM, whereas the effects of decorin peptide were observed over a range of 6.0–30 μM (Table 2). Collectively, these results suggest that decorin regulates clotting by binding to fibrinogen through the N-terminal region of the core protein. The observed differences in the regulatory potency of the intact core protein (45 kDa) and peptide (5 kDa), which bind to fibrinogen with similar affinity (49), support a mechanism in which decorin sterically influences fibrin assembly.

**Decorin Modulates Fibrin Assembly and Structure**—Conversely, we addressed an alternative possibility that decorin alters fibrin clotting by directly inhibiting thrombin activity. Hydrolysis of the chromogenic synthetic substrate S-2238 by thrombin was monitored in the absence or presence of decorin core protein, the decorin N-terminal domain, or hirudin (data not shown). As anticipated, the anticoagulant protein hirudin inhibited thrombin activity. However, neither decorin core protein nor peptide had an effect on the rate of chromophore release. Thus, it appears unlikely that decorin core protein attenuates thrombin-induced fibrin clotting through an effect on thrombin activity.

Decorin binds to the D regions of fibrinogen that participate in self-association through a number of constitutively interactive sites. To address the possibility that decorin limits fibrin assembly into protofibrils, we used SDS-PAGE to observe the cross-linking of γ-chains during clotting. The rapid cross-linking of fibrin γ-chains depends on the ability of the “a” polymerization sites in the D regions to recognize thrombin-exposed “A” sites emerging from the E regions as well as on the alignment of the C-terminal factor XIIIa cross-linking sites (referred to as γXL) (54, 55). Clots formed over a 5-min period from 2.0 μM fibrinogen in the absence or presence of 0.4–2.0 μM decorin core protein each exhibited complete γ-chain cross-linking into dimers (Fig. 2). Thus, decorin-fibrinogen interaction does not disrupt the D-E or γXL-γXL interactions within protofibrils.

To observe whether decorin influences the lateral assembly of protofibrils into fibers, we examined the fibrin clots by SEM (Fig. 3). Fig. 3A shows that fibers derived from fibrinogen were subtly curving and cable-like, ranging in diameter from 40 to 272 nm (mean = 146 nm). In striking contrast, clots formed in the presence of decorin comprised a curling, tangled arrangement of relatively thin fibers (Fig. 3, B and C). A 1:5 ratio of decorin to fibrinogen resulted in decreased fiber diameters spanning 17–105 nm (mean = 52 nm). At a 1:1 ratio, fiber girth was shifted further downward from 12 to 69 nm (mean = 35 nm). Altogether, these images demonstrate that decorin regulates clot structure by attenuating the lateral assembly of protofibrils into fibers.

**Decorin Regulates Clotting through Zn$^{2+}$-dependent Interactions**—In our previous studies, we demonstrated that the binding of the N-terminal region of decorin to fibrinogen is Zn$^{2+}$-dependent (47, 49). To determine whether the effect of decorin on fibrin clot formation shows a similar Zn$^{2+}$ dependence, we compared the effects of Zn$^{2+}$-charged and EDTA-treated forms of decorin on clot development (Fig. 4). Relative to fibrinogen alone, fibrinogen mixed with Zn$^{2+}$-charged decorin clotted at a slower rate and yielded a less turbid clot (Table 3), yet fibrinogen incubated with or without EDTA-treated decorin clotted at similar rates and exhibited equivalent final turbidities. Previous studies showed that clotting slows in the presence of EDTA, which chelates Zn$^{2+}$ and Ca$^{2+}$ ions, which, upon binding to the D regions, increase the polymerization rate and induce thicker fibers (56, 57). Our results suggest that the treatment of decorin with EDTA disrupts the confor-

---

**TABLE 1**

Quantitative effects of increasing decorin core protein concentrations on fibrin clotting

| Decorin conc (μM) | Rate (milli-absorbance/min) | Final absorbance |
|-------------------|-----------------------------|------------------|
| 0 μM              | 90.0 ± 4.3                  | 0.206 ± 0.005    |
| 0.2 μM            | 61.8 ± 4.3 (p < 0.01)       | 0.160 ± 0.004 (p < 0.01) |
| 0.4 μM            | 55.0 ± 3.0 (p < 0.002)      | 0.145 ± 0.003 (p < 0.002) |
| 1.0 μM            | 38.8 ± 4.3 (p < 0.0003)     | 0.120 ± 0.002 (p < 0.0003) |
| 2.0 μM            | 34.5 ± 2.5 (p < 0.0006)     | 0.113 ± 0.001 (p < 0.0006) |

**TABLE 2**

Quantitative effects of increasing decorin peptide concentrations on fibrin clotting

| Decorin N-terminal domain conc (μM) | Rate (milli-absorbance/min) | Final absorbance |
|-------------------------------------|-----------------------------|------------------|
| 0 μM                                | 110 ± 6                     | 0.240 ± 0.01     |
| 2.0 μM                              | 102 ± 3                     | 0.230 ± 0.01     |
| 6.0 μM                              | 91 ± 3                      | 0.220 ± 0.01 (p < 0.03) |
| 12 μM                               | 80 ± 2 (p < 0.05)           | 0.213 ± 0.002 (p < 0.01) |
| 30 μM                               | 70 ± 3 (p < 0.002)          | 0.197 ± 0.005 (p < 0.01) |
Decorin Modulates Fibrin

Fibrinogen was diluted to a concentration of 1.2 μM in 20 mM Hepes, 150 mM NaCl, 0.1% (w/v) CHAPS, 5 mM κ-aminocaproic acid, and 200 mM EDTA (pH 7.4) alone (lane 1) or with 0.26 μM decorin core protein previously dialyzed with Zn2+ ions (lane 2). Alternatively, fibrinogen was diluted to a concentration of 1.2 μM in 20 mM Hepes, 150 mM NaCl, 0.1% (w/v) CHAPS, 5 mM κ-aminocaproic acid, and 200 mM EDTA (pH 7.4) alone (lane 3) or with 0.26 μM EDTA-treated decorin (lane 4). The clotting reactions were monitored following the addition of thrombin as described in the legend of Fig. 1. The means ± S.D. of four polymerization curves are given in Table 3.

TABLE 3
Quantitative effects of Zn2+-charged or EDTA-treated decorin on fibrin clotting

| Decorin conc | Conditions | Rate (milli-absorbance/min) | Final absorbance |
|--------------|------------|----------------------------|-----------------|
| 0 μM         | Zn2+ ions  | 69 ± 6                     | 0.145 ± 0.001   |
| 0.26 μM      | Zn2+ ions  | 24 ± 3                     | 0.122 ± 0.005   |
| 0 μM         | EDTA       | 24 ± 3                     | 0.128 ± 0.006   |
| 0.26 μM      | EDTA       | 24 ± 2                     |                 |

These images showed that the corresponding mean fiber girths were 32 and 34 nm. Likewise, the range of fiber diameters spanned 8–60 nm and 8–76 nm, respectively. As with clot assembly conditions that prevent the binding of decorin to fibrinogen inhibit the effects on fibrin structure.

Our results suggest that the ability of decorin to modulate clot development and structure stems from the Zn2+-dependent interaction of decorin with fibrinogen. We have also provided evidence that decorin sterically attenuates the assembly of fibrin fibers. Such an effect might require the retention of decorin on the D region of fibrin. To determine whether decorin becomes incorporated into the fibrin matrix, clots were obtained from fibrinogen incubated alone or with decorin previously introduced to Zn2+ ions or EDTA. The soluble and matrix components from the clots were separated by centrifugation, fractionated by SDS-PAGE, and subjected to Western blotting to detect decorin (Fig. 6). EDTA-treated decorin was specifically detected in the supernatant of the clots (lane 2). Conversely, Zn2+-charged decorin precipitated with the fibrin matrix (lane 5). These results support the hypothesis that decorin retained by a Zn2+-dependent interaction with the D regions regulates the lateral growth of fibrin fibers during clotting.

Decorin Enhances Fibrin Clearance—One possible downstream impact of the regulation of clot structure by decorin is an altered rate of fibrin clearance. Fibrin D regions participate as cofactors of plasmin release through low affinity binding sites for t-PA and plasminogen (58, 59). After 2 h of clot development in the absence or presence of decorin core protein, the rate of decreasing fibrin clot turbidity was observed following overlay with a solution containing t-PA and plasminogen. To account for differences in the initial turbidity of fibrin clots containing or lacking decorin, measurements were converted to a percentage of clot lysis (Fig. 7A) (53). Relative to fibrin alone, fibrin with decorin was initially cleaved at a 48% faster rate and reached completion 61 min earlier. Indeed, curving thin fibers containing decorin were degraded in a shorter time span, perhaps because of an earlier onset of plasminogen activation. In

FIGURE 4. Effects of Zn2+-charged and EDTA-treated forms of decorin on fibrin clotting. Fibrinogen was diluted to a concentration of 1.2 μM in 20 mM Hepes, 150 mM NaCl, 0.1% (w/v) CHAPS, 5 mM κ-aminocaproic acid, 5 mM CaCl2, and 20 μM ZnCl2 (pH 7.4) alone (lane 5) or with 0.26 μM decorin core protein previously dialyzed with Zn2+ ions (lane 6). Alternatively, fibrinogen was diluted to a concentration of 1.2 μM in 20 mM Hepes, 150 mM NaCl, 0.1% (w/v) CHAPS, 5 mM κ-aminocaproic acid, and 200 μM EDTA (pH 7.4) alone (lane 7) or with 0.26 μM EDTA-treated decorin (lane 8). The clotting reactions were monitored following the addition of thrombin as described in the legend of Fig. 1. The means ± S.D. of four polymerization curves are given in Table 3.

FIGURE 3. SEM images of fibrin clots developed with or without decorin. Clots were derived from 1.2 μM fibrinogen alone (A) or with 0.26 μM (B) or 1.2 μM (C) decorin in 20 mM Hepes, 150 mM NaCl, 0.1% (w/v) CHAPS, 5 mM κ-aminocaproic acid, 5 mM CaCl2, and 20 μM ZnCl2 (pH 7.4). Clotting was induced by the addition of 0.4 units/ml thrombin and allowed to proceed at −25 °C for 3 h. All images were acquired at an original magnification of ×16,000. The images shown appear similar to others obtained from the same or separate microscopy preparations.
tance in hemostasis/thrombosis, inflammation, and wound repair (60). Decorin regulates collagen fibrillogenesis through binding to collagen and limiting the lateral assembly of fibers (30, 34–38). Our previous study demonstrated the binding of decorin to fibrinogen D regions (49). Hence, we began here by exploring for a parallel function of decorin as a modulator of fibrin clotting. Indeed, this study has revealed that decorin binding to fibrinogen can regulate fibrin fiber assembly, structure, and clearance.

Fibrin clotting curves comprise up to three regions in which the turbidity corresponding to fiber accumulation lags, rises, and plateaus (4). Toward the end of the lag phase, protofibrils begin to associate laterally into fibers, and thereafter, the rate of rising turbidity corresponds to the rate of lateral aggregation (61, 62). We observed a lag prior to fiber assembly in solutions containing EDTA, but no lag in solutions containing Zn\(^{2+}\) and Ca\(^{2+}\) ions. A delay of ~1 min between the addition of thrombin and the time of the first turbidity measurement may have prevented the detection of a lag in clotting with divalent cations. Unlike the curve from clotting fibrinogen alone, the inclusion of decorin core protein resulted in an initial turbidity value of >0. This observation suggests that decorin could increase the rate of initiation of protofibrils, thereby favoring the formation of a large number of thin fibers (62). Alternatively, the binding of decorin to the D region could sterically limit the lateral assembly of fibers.

Further mechanistic insight can be gleaned by comparing the clotting and structural characteristics of certain variant forms of fibrinogen with those of normal fibrinogen with or without decorin. Relative to normal fibrinogen, the mutations γY363A and γD364A, affecting the a sites for protofibril formation, result in a longer lag and reduced rate of clotting (63). Although the clotting times are also prolonged, the final turbidity eventually reaches that of normal fibrin. This is unlike the effects of decorin, which decreased the rate and final turbidity without altering the lag or clotting time. Because decorin influences clotting much differently compared with γY363A and γD364A, we suggest that decorin does not interfere with the a sites driving protofibril assembly.

Our data show that cross-linked γ-chain dimers form rapidly in the presence or absence of decorin. If decorin binding to the D region had caused misalignment of the D-D interface, we might have observed a reduced rate of γ-chain cross-linking similar to the effect of the abnormal glycosylation of γAsn\(^{308}\) (64, 65). Alternatively, the study of dysfibrinogen Tokyo II γR275C showed that D-D misalignment does not always cause decreased fibrin or fibrinogen γ-chain cross-linking (55). Comparing SEM images, we found that the fibrin network formed with decorin differed from that reported for Tokyo II fibrin. Collectively, decorin does not detectably disrupt a site, D-D, or γXL-γXL interactions required for normal protofibril assembly and stabilization.

**FIGURE 5.** SEM images of clots developed in the presence of EDTA. Clots were developed from 1.2 μM fibrinogen alone (A) or with 0.26 μM EDTA-treated decorin (B) in 20 mM Hepes, 150 mM NaCl, 0.1% (w/v) CHAPS, 5 mM γ-aminocaproic acid, 5 mM CaCl\(_2\), 200 μM EDTA, and 20 μM ZnCl\(_2\) (pH 7.4). Clots were developed and prepared for imaging as described in the legend of Fig. 2. All images shown were acquired at an original magnification of ×16,000 and appear similar to those obtained from other microscopy preparations.

**FIGURE 6.** Zn\(^{2+}\)-dependent incorporation of decorin core protein into the fibrin matrix. Clots developed from the same mixtures utilized to follow the progress of clotting (shown in Fig. 3) were fractionated by centrifugation. Lanes 1, 3, and 5 correspond to the resuspended pellets comprising the clot matrix (M), whereas lanes 2, 4, and 6 represent the clot supernatants (S). The clot fractions were boiled in SDS-PAGE sample loading buffer with 2-mercaptoethanol. Pairs of lanes were loaded onto 4% and 8% gels with clot components (lanes 1 and 2) or buffer only (lanes 3 and 4), and decorin with Zn\(^{2+}\) ions (lanes 5 and 6). Lanes 7 and 8 contained purified decorin core proteins and molecular mass standards, respectively. To detect decorin core proteins on Western blots, membranes were incubated initially for 30 min with PR2 antiserum diluted 2000-fold and then for 20 min with alkaline phosphatase-conjugated goat anti-rabbit IgG diluted 5000-fold in HBS containing 0.1% (w/v) blocking solution. Replicate experiments produced equivalent results. N/A, not applicable.

**DISCUSSION**

The D regions present many major functional sites on fibrinogen for cellular and molecular interactions that are of impor-
SEM images confirmed that the relatively reduced turbidity of fibrin clots formed in the presence of decorin arises from a network of thinner fibers. Among characterized dysfibrinogens, those exhibiting solely impaired lateral assembly seem to be relatively rare (60). Fibrinogen Lima bears an extra oligosaccharide moiety on the α-chain in the coiled-coil segment proximal to the βC domain, implicated in protofibril assembly (7, 66). Similar to the effect of decorin, the Lima-type glycosylation exerts no effect on the lag, yet decreases the rate and turbidity. Furthermore, curvature associated with thin Lima fibrin fibers has been suggested to reflect altered molecular packing (66). Decorin could perturb protofibril-protofibril interactions by binding to the coiledcoil or nearby segments 330–375, proposed to participate in fiber assembly (7).

Decorin modulated fibrin clotting in a concentration-dependent fashion. The maximum regulatory effect on the clotting of 2.0 μM fibrinogen occurred at a concentration of 1.0 μM decorin core protein in which less than half of the D regions were occupied. To approach the effect of decorin core protein (42–45 kDa), an excess of 30 μM decorin peptide (5 kDa) was necessary. We have shown previously that intact decorin proteoglycan and peptide bind to fibrinogen with similar affinities, \( K_D = 6.8 \times 10^{-7} \) and 3.0 \( \times 10^{-7} \) M\(^2\), respectively (49). Hence, the greater decorin peptide concentration required to regulate clotting does not seem to reflect a lower affinity of fibrinogen, but instead suggests that steric hindrance plays a key role in the decorin core protein effect on fibrin clot assembly. We found previously through gel filtration chromatography that Zn\(^{2+}\) ions induce the self-association of decorin peptides into multimeric forms (49). Thus, to sterically attenuate fibrin assembly, decorin core protein or a multimeric form of decorin peptide must be bound to the D region.

Our data support the hypothesis that the direct Zn\(^{2+}\)-dependent binding of decorin to fibrinogen represents the mechanism of fiber growth regulation. We found that Zn\(^{2+}\)-charged (but not EDTA-treated) forms of decorin precipitated with the fibrin clot. Likewise, decorin exposed to EDTA lacked observable effects on clot development and fibrin structure. We demonstrated previously (49) through the inhibitory effect of EDTA that decorin-fibrinogen association requires Zn\(^{2+}\) ions at a physiological concentration (20 μM) (67).

Another previous study from our laboratory showed that decorin is a Zn\(^{2+}\)-metalloprotein through comparative CD spectra that revealed EDTA-induced changes in the peptide backbone conformation (47). Thus, EDTA likely inhibits decorin-fibrinogen interaction by inducing structural changes in the N-terminal region of the core protein of decorin. The fibrin fibers formed in the presence of EDTA with or without decorin exhibited a lower mean diameter and range than those formed in the presence of Ca\(^{2+}\) and Zn\(^{2+}\) ions. Although these divalent cations have been shown to bind to fibrinogen D regions and to increase fiber girth (56, 57, 68), decorin bound to the D regions was counter-regulatory in as much as thinner fibers formed. It is then tempting to speculate that perhaps decorin binds to the D region nearby one of the cation-binding sites regulating the lateral assembly of fibrin fibers. In this case, divalent cations would also be required by fibrinogen for binding to decorin.

Several characteristics of the fibrin network can contribute to the rate of fibrinolysis, including interactions with molecular or ionic regulators of fiber diameter, fibrinogen conformation, and the extent of γ-chain cross-linking (69). A previous study has revealed that, regardless of the conditions favoring thinner fibers, increased ionic strength or thrombin concentration, such clots undergo delayed t-PA-dependent fibrinolysis (70). Moreover, thin fibrin networks associated with certain dysfibrinogens (e.g. Dusart or Chapel Hill III), myeloma, nephrotic disease, diabetes, or myocardial infarction require greater time for clearance compared with clots of thick fibers (53, 71–75). Although the lysis of individual thin fibers has been shown to occur more rapidly that that of thicker ones, fibrin conformation and spatial density more than diameter alone influence lysis rate (76). Fibrin formed in the absence or presence of decorin was composed mainly of straight, thick, stationary fibers or curly, thin, flexible fibers, respectively. Relative to fibrin, we discovered that the rate of fibrinolysis induced by t-PA activation of plasminogen was increased in the fibrin-decorin network. Although fibrin formed in the absence or presence of decorin was degraded by pre-activated plasmin at similar rates, this would not have been predicted for thinner fibrin fibers. Decorin may favor the formation of thinner fibers with altered t-PA or plasminogen binding characteristics, thereby promoting t-PA-dependent proteolysis of thin fibers anticipated to be lysis-resistant.

The results presented here demonstrate that decorin core protein can modulate fibrin clot assembly, structure, and degradation in an in vitro experimental system using purified proteins. The physiological relevance of these observations appears...
unclear because the decorin-deficient mouse does not exhibit any obvious coagulation defects. However, a recent study in our laboratory has shown that these mice are more vulnerable to Staphylococcus aureus-induced experimental sepsis compared with wild-type mice and that this susceptibility is associated with a hypercoagulant state in the challenged decorin-deficient mice. Thus, decorin appears to play an important role in maintaining the hemostatic balance when this is challenged by bacterial infections.

Decorin has been shown to activate the thrombin inhibitor heparin cofactor II (77). This study has illuminated a new role of decorin as a regulator of fibrinogen function. Thinner flexible fibrils reportedly serve as optimum scaffolds for macrophage migration, yet are poor substrates for fibroblast adhesion (78, 79). By favoring these fibrin fiber types, decorin could regulate the timing of cellular events during wound healing. Given that the interactions with collagen and fibrinogen occur on distinct regions of the core protein, decorin perhaps functions as a molecular bridge stabilizing the thrombus against embolism. Alternatively, decorin in a ruptured plaque could potentially provide adhesive sites for fibrinogen participating in the deposition of a fibrin network or the adhesion of leukocytes and platelets. Decorin inhibits fibrosis in animal models of response-to-injury-mimicking diseases such as intimal hyperplasia, pulmonary fibrosis, glomerulonephritis, corneal inflammation, and myocardial infarction (80–84). Although this effect of decorin has been thought to stem from the ability of the core protein to bind to transforming growth factor-β and to modulate its fibrosis-promoting activity, effects on fibrin organization could also contribute. Future studies are warranted to determine the importance of decorin interaction with fibrinogen and regulation of fibrin organization in wound repair, vascular disease, and sepsis.

Acknowledgment—We thank Tom Stephens (Texas A&M University Microscopy and Imaging Center) for acquiring the SEM images of our fibrin clots.

REFERENCES

1. Mosesson, M. W., Siebenlist, K. R., and Meh, D. A. (2001) Ann. N. Y. Acad. Sci. 950, 1–133
2. Blomback, B., Hessel, B., Hogg, D., and Therikildes, L. (1978) Nature 275, 501–505
3. Mullin, J. L., Gorkun, O. V., Binnie, C. G., and Lord, S. T. (2000) J. Biol. Chem. 275, 25239–25246
4. Hantgan, R. R., and Herrmans, J. (1979) J. Biol. Chem. 254, 11272–11281
5. Salah, J. R., and Gribbin, R. H. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 1872–1876
6. Spragg, G., Everse, S. J., and Doolittle, R. F. (1997) Nature 389, 455–462
7. Doolittle, R. F., Yang, Z., and Mochalin, I. (2001) Ann. N. Y. Acad. Sci. 936, 31–43
8. Mosesson, M. W., Siebenlist, K. R., Amrani, D. L., and DiOrio, J. P. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 1113–1117
9. Takagi, T., and Doolittle, R. F. (1974) Biochemistry 13, 750–756
10. Folk, J. E., and Finlayson, J. S. (1977) Adv. Protein Chem. 31, 1–133
11. Greiling, D., and Clark, R. A. (1997) J. Cell Sci. 110, 861–870
12. Clark, R. A. (2001) Ann. N. Y. Acad. Sci. 936, 355–367
13. Jarvelainen, H. T., Kinsella, M. G., Wight, T. N., and Sandell, L. J. (1991) J. Biol. Chem. 266, 23274–23281
14. Kaji, T., Sakurai, S., Yamamoto, C., Fujisawa, Y., Yamagishi, S., Yamamoto, H., Kinsella, M. G., and Wight, T. N. (2001) Bioll. Pharm. Bull. 27, 1763–1768
15. Jarvelainen, H. T., Iruela-Arispe, M. L., Kinsella, M. G., Sandell, L. J., Sage, E. H., and Wight, T. N. (1992) Exp. Cell Res. 203, 395–401
16. Radhakrishnamurthy, B., Tracy, R. E., Dalferes, E. R., Jr., and Berenson, G. S. (1998) Exp. Mol. Pathol. 65, 1–8
17. Merrilees, M. J., Beaumont, B., and Scott, L. J. (2001) Coron. Artery Dis. 12, 7–16
18. Riessen, R., Isner, J. M., Blessing, E., Loushin, C., Nikol, S., and Wight, T. N. (1994) Am. J. Pathol. 144, 962–974
19. Gutierrez, P., O’Brien, K. D., Ferguson, M., Nittkar, S. T., Alpers, C. E., and Wight, T. N. (1997) Cardiovasc. Pathol. 6, 271–278
20. Evanko, S. P., Raines, E. W., Ross, R., Gold, L. I., and Wight, T. N. (1998) Am. J. Pathol. 152, 533–546
21. Jarrell, J. E., and Finlayson, W. S. (1999) Atherosclerosis 146, 299–308
22. Lin, H., Wilson, J. E., Roberts, C. R., Horley, K. J., Winters, G. L., Costanzo, M. R., and Macmanus, B. (1996) J. Heart Lung Transplant. 15, 1233–1247
23. Koldodige, F. D., Burke, A. P., Farb, A., Weber, D. K., Kutys, R., Wight, T. N., and Virmani, R. (2002) Arterioscler. Thromb. Vasc. Biol. 22, 1642–1648
24. Strom, A., Ahleqvist, E., Frenzen, A., Heinegard, D., and Hultgardh-Nilsson, A. (2004) Histol. Histopathol. 19, 337–347
25. Fischer, J. W., Steitz, S. A., Johnson, P. Y., Burke, A., Koldodige, F., Virmani, R., Giachelli, C., and Wight, T. N. (2004) Arterioscler. Thromb. Vasc. Biol. 24, 2391–2396
26. Shin, R. A., Parthasarathy, N., San Antonio, J. D., Church, F. C., and Wagner, W. D. (2000) J. Biol. Chem. 275, 18085–18092
27. Whinna, H. C., Choi, H. U., Rosenberg, L. C., and Church, F. C. (1993) J. Biol. Chem. 268, 3920–3924
28. Iozzo, R. V. (1997) Nat. Rev. Biochem. Mol. Biol. 32, 141–174
29. Hocking, A. M., Shimamura, T., and McQuillan, D. J. (1998) Matrix Biol. 17, 1–19
30. Danielson, K. G., Barbui, I., Holmes, D. F., Graham, H., Kadler, K. E., and Iozzo, R. V. (1997) J. Cell Biol. 136, 729–743
31. Svensson, L., Ass佐di, A., Reinhold, F. P., Fassler, R., Heinegard, D., and Oldberg, A. (1999) J. Biol. Chem. 274, 9636–9647
32. Chakravarti, S., Magnusson, T., Lass, J. H., Jepsen, K. J., LaMantia, C., and Carroll, H. (1998) J. Cell Biol. 141, 1277–1286
33. Koldodige, F. D., Burke, A. P., Farb, A., Weber, D. K., Kutys, R., Wight, T. N., and Virmani, R. (2002) Arterioscler. Thromb. Vasc. Biol. 22, 1642–1648
34. Schonherr, E., Hausser, H., and Kresse, H. (1991) Matrix Biol. 10, 241–248
35. Timpl, R., Giachelli, C., and Wight, T. N. (2004) J. Biol. Chem. 279, 23274–23281
36. Schonherr, E., Hausser, H., and Kresse, H. (1991) Matrix Biol. 10, 241–248
Decorin Modulates Fibrin

47. Yang, V. W.-C., LaBrenz, S. R., Rosenberg, L. C., McQuillan, D., and Höök, M. (1999) J. Biol. Chem. 274, 12454–12460
48. Liu, J., Laue, T. M., Choi, H. U., Tang, L. H., and Rosenberg, L. (1994) J. Biol. Chem. 269, 28366–28373
49. Dugan, T. A., Yang, V. W.-C., McQuillan, D. J., and Höök, M. (2003) J. Biol. Chem. 278, 13655–13662
50. Ramamurthy, P., Hocking, A. M., and McQuillan, D. J. (1996) J. Biol. Chem. 271, 19578–19584
51. Esko, J. D., Stewart, T. E., and Taylor, W. H. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 3197–3201
52. Hogan, K. A., Gorkun, O. V., Lounes, K. C., Coates, A. I., Weisel, J. W., Hantgan, R. R., and Lord, S. T. (2000) J. Biol. Chem. 275, 17778–17785
53. Mullin, J. L., Norfolk, S. E., Weisel, J. W., and Lord, S. T. (2001) Ann. N. Y. Acad. Sci. 936, 331–334
54. Kanaide, H., and Shainoff, J. R. (1975) J. Lab. Clin. Med. 85, 382–387
55. Mosesson, M. W., Siebenlist, K. R., DiOrio, J. P., Hainfeld, J. F., and Wall, J. S. (1995) J. Clin. Investig. 96, 1053–1058
56. Scully, M. F., and Kakkar, V. V. (1983) Thromb. Res. 30, 297–300
57. Boyer, M. H., Shainoff, J. R., and Ratnoff, O. D. (1972) Blood 39, 574–597
58. Voskuilen, M., Vermond, A., Weisel, J. W., and Lord, S. T. (2000) J. Biol. Chem. 275, 17778–17785
59. Yoshino, H., Gorkun, O. V., and Lord, S. T. (1997) Blood 90, 147–153
60. Marchi, R., Arocha-Pinango, C. L., Nagy, H., Matsuda, M., and Weisel, J. W. (2005) J. Thromb. Haemostasis 3, 4157–4163
61. Keller, M. A., Martinez, J., Baradet, T. C., Nagaswami, C., Chernysh, I. N., Borowski, M. K., Surrey, S., and Weisel, J. W. (2005) Blood 105, 3162–3168
62. Weisel, J. W., and Nagaswami, C. (1992) Biochem. J. 283, 187–191
63. Cote, H. C., Lord, S. T., and Pratt, K. P. (1998) Blood 92, 2195–2212
64. Keller, M. A., Martinez, J., Baradet, T. C., Nagaswami, C., Chernysh, I. N., Borowski, M. K., Surrey, S., and Weisel, J. W. (2005) Blood 105, 3162–3168
65. Weisel, J. W., and Nagaswami, C. (1992) Biochem. J. 283, 29596–29601
66. Yamazumi, K., Shimura, K., Maekawa, H., Muramatsu, S., Terukina, S., and Matsuda, M. (1996) Blood 90, 557–559
67. Okumura, N., Gorkun, O. V., and Lord, S. T. (1997) J. Biol. Chem. 272, 14157–14163
68. Marchi, R., Arocha-Pinango, C. L., Nagy, H., Matsuda, M., and Weisel, J. W. (2004) J. Thromb. Haemostasis 2, 940–948
69. Yonekawa, O., Voskuilen, M., and Nieuwenhuizen, W. (1987) J. Biol. Chem. 262, 5944–5946
70. Okumura, N., Gorkun, O. V., and Lord, S. T. (2004) Blood 103, 4157–4163
71. Wada, Y., and Lord, S. T. (1994) Blood 84, 3709–3714
72. Carr, M. E., Jr., and Alving, B. M. (1995) Blood Coagul. Fibrinolysis. 6, 567–573
73. Collet, J. P., Park, D., Lesty, C., Soria, J., and Soria, C. (1999) Thromb. Haemostasis 82, 1482–1489
74. Brownlee, M., Vlassara, H., and Cerami, A. (1983) Diabetes 32, 680–684
75. Fatih, K., Silveira, A., Tornvall, P., Karpe, F., Blomback, M., and Hamsten, A. (1996) Thromb. Haemostasis 76, 535–540
76. Collet, J. P., Park, D., Lesty, C., Soria, J., Soria, C., Montalescort, G., and Weisel, J. W. (2000) Arterioscler. Thromb. Vasc. Biol. 20, 1354–1361
77. Delorme, M. A., Xu., J., Berry L., Mitchell, L., and Andrew, M. (1998) Thromb. Res. 90, 147–153
78. Ciano, P. S., Colvin, R. B., Dvorak, A. M., McDonagh, J., and Dvorak, H. F. (1986) Lab. Investig. 54, 62–70
79. Amrani, D. L., DiOrio, J. P., and Delmote, Y. (2001) Ann. N. Y. Acad. Sci. 936, 566–579
80. Nili, N., Cheema, A. N., Giordano, F. J., Barolet, A. W., Babaei, S., Hickey, R., Eskandarian, M. R., Smeets, M., Butany, J., Pasterkamp, G., and Strauss, B. H. (2003) Am. J. Pathol. 163, 869–878
81. Klob, M., Margetts, P. J., Galt, T., Sime, P. J., Xing, Z., Schmidt, M., and Gautlie, J. (2001) Am. J. Respir. Crit. Care Med. 163, 770–777
82. Isaka, Y., Brees, D. K., Ikegaya, K., Kaneda, Y., Imai, E., Noble, N. A., and Border, W. A. (1996) Nat. Med. 2, 418–423
83. Schonherr, E., Sunderkotter, C., Schaefer, L., Thanos, S., Grassel, S., Oldberg, A., Iozzo, R. V., Young, M. F., and Kresse, H. (2004) J. Vasc. Res. 41, 499–508
84. Weis, S. M., Zimmerman, S. D., Shah, M., Covell, J. W., Omens, J. H., Ross, J., Dalton, N., Jones, Y., Reed, C. C., Iozzo, R. V., and McCulloch, A. D. (2005) Matrix Biol. 24, 313–324