Selective Expression and Processing of Biglycan during Migration of Bovine Aortic Endothelial Cells

THE ROLE OF ENDOGENOUS BASIC FIBROBLAST GROWTH FACTOR*

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Repair of the vascular luminal surface after injury requires a controlled endothelial cell response that includes cell migration, proliferation, and remodeling of the extracellular matrix. These cellular processes are modulated by growth factors that are released or activated following cell injury. When endothelial cell migration is stimulated in response to monolayer wounding in vitro, cells increase synthesis of small leucine-rich dermatan sulfate proteoglycans (PGs) (Kinsella, M. G., and Wight, T. N. (1986) J. Cell Biol. 102, 679–687). However, the identity of the PGs that are increased during cell migration and the factors that affect this modulation have not been identified. We now report that basic fibroblast growth factor (bFGF) is responsible for the transient increase of $[^{35}\text{S}]$sulfate incorporation into PGs following monolayer wounding. SDS-polyacrylamide gel electrophoresis analysis revealed that bFGF-treated and wounded cultures increase both biglycan core protein synthesis and biglycan proteolytic processing, which results in the accumulation of a ~20-kDa N-terminal biglycan fragment in the culture media. Biglycan RNA steady-state levels also selectively increase 2- to 3-fold after wounding or bFGF treatment. Finally, immunocytochemical staining localizes biglycan to the tips and edges of lamellopodia on migrating cells, indicating that biglycan is found at loci at which the formation and dissolution of adhesion plaques occurs, consistent with hypotheses that predict involvement of biglycan in the control of cell migration. Taken together, these results suggest that release of endogenous bFGF is primarily responsible for altered biglycan expression, synthesis, and proteolytic processing as endothelial cells migrate after wounding.

Migration and proliferation of endothelial cells and the subsequent establishment of a stable monolayer are critical events in the repair of injured vessels and in angiogenesis and vasculogenesis during development, tumor growth, and tissue repair. Cell migration and proliferation are controlled by multiple factors, such as FGF and transforming growth factor-$\beta$ (1, 2), and cell adhesive interactions, mediated by cell surface receptors such as integrins (3). Thus, initiation of cell migration may involve a change in cell-matrix interaction characterized by extracellular matrix remodeling and altered matrix receptor expression, which are processes directed in part by growth factors.

Proteoglycans are a heterogeneous group of protein families that bear anionic glycosaminoglycan (GAG) chains covalently bound to core proteins. These molecules are prominent constituents of both the extracellular matrix and the cell surface, where they are proposed to play roles in cell adhesion, growth factor interactions, and matrix assembly (4, 5). Members of the small leucine-rich PG family, such as biglycan and decorin, which bear dermatan or chondroitin sulfate side chains, bind fibrillar proteins, such as collagen (6, 7), and may regulate fibrillogenesis (8). In addition, biglycan and decorin may affect cell migration both by modulating interactions of cell surface receptors with their matrix ligands (9, 10), and by influencing growth factor availability and function (5).

We have previously reported that the induction of migration in wounded endothelial cell monolayers is accompanied by increased dermatan sulfate PG synthesis, which is associated with cells at the wound edge (11). The principal dermatan sulfate PG synthesized by confluent cultured aortic endothelial cells is biglycan (12, 13), consistent with immunochemical and in situ hybridization studies of endothelia in vessels (14, 15). However, decorin expression, which is not detectable in monolayers of aortic endothelial cells (13), is induced in concert with type I collagen when cells undergo sprouting and tube formation in vitro (16). Thus, decorin and biglycan expression are differentially regulated as endothelial cells migrate, proliferate, or alter their phenotype. Although changes in the expression of small leucine-rich PGs may affect extracellular matrix assembly, cellular adhesion, or growth factor utilization that is critical to cell migration, the factors that control these changes are poorly understood. bFGF is clearly a candidate for such a role, as it alters migration, proliferation, and matrix protein deposition by endothelial cells (2), and is released from cultured monolayers after wounding in vitro (17). Therefore, we have investigated the role of endogenous bFGF release as a mechanism that may control the expression of PGs after wounding of endothelial monolayers in vitro. We find that increased expression and proteolytic processing of biglycan, which is present on the lamellipodia of migrating cells, is selectively induced by the release of endogenous bFGF after multiscratch wounding of monolayers, suggesting that biglycan metabolism may be involved in the control of cell migration.

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eluting bound macromolecules in urea buffer with 3 M NaCl. Portions of (urea buffer), washing with... fractions of individual PG bands. Thus, mixtures of PG, prepared for 5-bromo-4-chloro-3-indolyl phosphate (Sigma).

Secondary antibodies, bands that bound primary antibodies were visualized... (Bio-Rad Laboratories). Nitrocellulose membranes (Bio-Blot-NC, Costar, Cambridge, MA) or Zetaprobe GT (Bio-Rad Laboratories), and UV-cross-linked (Stratagene Cloning Systems, La Jolla, CA). Prior to hybridization, filters were prehybridized for at least 2 h at 42 °C in a solution containing 50% v/v formamide (Life Technologies, Inc.), 6 × SSPE (1 × SSPE = 0.15 M NaCl, 0.2 mM NaH2PO4, and 0.02 M tetrasodium EDTA), 5 × Denhardt’s solution (1 × Denhardt’s = 0.02% Ficoll, 0.02% polyvinylpyrrolidone, and 0.02% bovine serum albumin), 0.5% SDS, 5% dextran sulfate (5 prime – 5 prime, Inc., Boulder, CO), and 0.1% formaldehyde in saline sodium citrate (Sigma). Hybridization... labeled cDNA probes (prepared as described below) were carried out at 42 °C in the same solution for at least 16 h, after which the filters were washed three times with 2 × SSPE/0.1% SDS at 42 °C, and twice with 0.3 × SSPE/0.1% SDS at 65 °C.

cDNA Probes—Several cDNAs were used as probes for PG RNA on Northern blots, including full-length human biglycan cDNA (P16) and full-length bovine decorin cDNA, provided by Dr. L. Fisher (27) and Dr. M. Young (28), respectively, of the NIDR, NIH, Bethesda, MD; human perlecan cDNA (HS-1) from Dr. R. V. Iozzo, Thomas Jefferson University, Philadelphia, PA (29), and human versican cDNA (C10) from Dr. E. Ruoslahti (30). Northern blots were normalized for loading by comparison to hybridization of bovine 28 S rRNA cDNA that was kindly provided by Dr. E. H. Sage, University of Washington, Seattle, WA. Sense and antisense cDNA Probes were 5'-P-labeled by nick translation (Life Technologies, Inc.) or random priming (Amersham), using 5'-[α-32P]dCTP (Amersham), as described previously (13), and used to hybridize with RNA on Northern blots prepared as described above. Autoradiographs were prepared by exposure on Kodak XAR2 film at ~70 °C and then developed. Quantitation of labeled bands was as described above for SDS-PAGE. Statistical analysis of sample means was by the two-tailed t test.

Immunocytochemistry—Cells were cultured on coverslips until confluent, at which time portions of some monolayers were removed with a razor blade fragment as described previously (11). Coverslips containing cells were washed twice with phosphate-buffered saline (PBS) and fixed for 15 min in freshly prepared 3% paraformaldehyde in PBS, pH 7.4, at 4 °C. After washing in PBS, cells on some coverslips were permeabized with 0.1% Triton X-100 in PBS, and coverslips were blocked in 10% calf serum in PBS and then in 2% goat serum (Sigma, for rabbit primary antibodies) or in 2% rabbit serum (for chicken primary antibodies). Prior to staining with LF-96, coverslips were treated with 0.05 unit/ml chondroitin ABC lyase in enriched Tris buffer (20) for 3 h at 37 °C. Cells were exposed to primary antibodies diluted in 10% calf serum at 4 °C overnight, at dilutions ranging from 1:50 to 1:500. Control coverslips were exposed to PBS, normal rabbit serum (Zymed Laboratories, Inc., So. San Francisco, CA), or normal chicken serum (Accurate Chemical and Scientific Corp., Westbury, NY), diluted to match the IgG concentrations in the diluted primary antibodies. As an additional control, preadsorption of diluted LF-96 with the N-terminal bovine biglycan peptide it was kindly provided by Dr. L. Fisher, NIDR, NIH was done at a concentration of 100 μg/ml, overnight at 4 °C, followed by centrifugation of the preadsorbed antibody. After washing with 10% bovine albumin in PBS, and then PBS alone, coverslips were treated with fluorescein isothiocyanate-labeled goat anti-rabbit (Zymed Laboratories, Inc.) or rabbit anti-chicken IgG (Zymed Laboratories, Inc.) diluted 1:200 in 10% calf serum, for 1 h, washed, and mounted in 10% glycerol for fluorescent microscopy.

RESULTS

Monolayer Wounding and Exogenous bFGF Cause Similar Transient Increases in Endothelial Cell PG Synthesis—Confluently

Selective Expression of Biglycan

Materials—Guadinine HCl, N-ethylmaleimide, phenylmethanesulfonyl fluoride, and chondroitin sulfate (type C) were from Sigma; XAR-2 film, 6-aminohexanoic acid, and benzamidine were from Eastman Kodak Co., Rochester, NY; chondroitin ABC lyase from ICN Pharmaceuticals, Costa Mesa, CA; DEAE-Sephacel and Sepharose CL-4B and CL-6B were from Pharmacia Biotech, Inc.; preincubated and 14C-labeled protein standards, glycine, SDS, acrylamide, methylenebisacrylamide, N,N,N',N'-tetramethylethylenediamine, and ammonium persulfate were from Life Technologies, Inc.; Triton X-100 was from Boehringer Mannheim Corp.; Na2[35S]SO4 (carrier-free) was from ICN Radiochemicals; and all cell culture supplies were from Life Technologies, Inc. Recombinant human basic FGF was provided courtesy of Dr. R. Majack, University of Colorado, Boulder, CO, or obtained from Intergen, Protein standards, Tris-HCl, pH 9.7, with 5 mM MgCl2, 10% methanol and 0.0375% SDS (24), and transferred to nitrocellulose (BioBlot-NC, Costar, Cambridge, MA) or Zetaprobe GT (Bio-Rad Laboratories), and UV-cross-linked (Stratagene Cloning Systems, La Jolla, CA). Prior to hybridization, filters were prehybridized for at least 2 h at 42 °C in a solution containing 50% v/v formamide (Life Technologies, Inc.), 6 × SSPE (1 × SSPE = 0.15 M NaCl, 0.2 M NaH2PO4, and 0.02 M tetrasodium EDTA), 5 × Denhardt’s solution (1 × Denhardt’s = 0.02% Ficoll, 0.02% polyvinylpyrrolidone, and 0.02% bovine serum albumin), 0.5% SDS, 5% dextran sulfate (5 prime – 5 prime, Inc., Boulder, CO), and 0.1% formaldehyde in saline sodium citrate (Sigma). Hybridization... labeled cDNA probes (prepared as described below) were carried out at 42 °C in the same solution for at least 16 h, after which the filters were washed three times with 2 × SSPE/0.1% SDS at 42 °C, and twice with 0.3 × SSPE/0.1% SDS at 65 °C.

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RESULTS

Monolayer Wounding and Exogenous bFGF Cause Similar Transient Increases in Endothelial Cell PG Synthesis—Confluently
ent cultures were exposed to increasing concentrations of bFGF for 12 h before pulsing for 2 h with [35S]sulfate to determine if bFGF altered PG synthesis. Incorporation of [35S]sulfate into PG is increased by bFGF in a dose-dependent manner (Fig. 1). When similarly labeled wounded cultures were compared at 2, 5, 24, and 48 h after wounding with confluent cultures treated with 10 ng/ml bFGF, a peak of incorporation ~2-fold higher than control was found (Fig. 2, A and B) after either treatment. This increase is apparent 24 h after wounding, in agreement with earlier studies (11), while the increase occurs by 5 h after bFGF treatment. bFGF treatment of cultures, like wounding, causes larger increases in PG synthesis over control in the media (Fig. 2, A and B) of the cultures than in the cell layers (not shown). In both culture treatments, the change in incorporation is transient and approaches control values by 48 h. Comparison of the relative proportions of [35S]sulfate incorporated into either chondroitin/dermatan sulfate or heparan sulfate with time after wounding (Fig. 3A) or bFGF treatment (Fig. 3B), indicates that chondroitin/dermatan sulfate is specifically increased after either treatment, while heparan sulfate is either decreased (after wounding) or unchanged (after bFGF treatment). These experiments suggest that both wounding and exogenous bFGF result in a transient, specific increase in synthesis of dermatan sulfate PG, the bulk of which is rapidly secreted to the culture media.

Neutralizing Anti-bFGF Antibodies Inhibit PG Synthesis by Migrating Cells—A neutralizing antibody against bFGF (number 2373-1 (18)) was added to confluent cultures prior to multiscratch wounding or addition of exogenous bFGF to determine if release of endogenous bFGF contributes to increased PG synthesis in wounded cultures (Fig. 4). Antibody-treated, control confluent, and bFGF-treated and wounded cultures were pulsed with radiolabeled media extracts from wounded and bFGF-treated cultures pulsed for 2 h at times for which increased incorporation had been demonstrated in preliminary experiments (10–12 h in bFGF-treated cultures and 25–27 h in multiscratch wounded cultures). Incorporation of [35S]sulfate into PG, normalized to cellular DNA, was increased over control levels in bFGF-treated and multiscratch wounded cultures. This increase was abrogated in cultures pretreated with anti-bFGF antibody. The ability of a neutralizing anti-bFGF antibody to block increased incorporation of [35S]sulfate into PG after multiscratch wounding or addition of exogenous bFGF provides direct evidence that increased synthesis of PG during cell migration after wounding involves the release of endogenous bFGF.

Identification of Biglycan as the Principal PG Showing Increased Synthesis after Monolayer Wounding or bFGF Treatment—[35S]Sulfate-labeled media extracts from wounded and bFGF-treated cultures pulsed for 2 h at various times after treatment were subjected to SDS-PAGE to determine the apparent Mr of specific PGs that show increased incorporation after stimulation (Fig. 5). As described previously (12, 13), several prominent [35S]sulfate-labeled bands are apparent after fluorography of the processed gel. The predominant PG band in media samples at all times after wounding or bFGF treatment ran with a Mr of 200–300,000 (arrowhead). Both the apparent Mr and relative amount of [35S]sulfate incorporated into this band increase transiently, with the differences appearing within 5 h after treatment. Control levels and the apparent size of the 200–300,000 Mr band are restored by
between 48 and 72 h after bFGF addition. Similar changes in the \( M_r \) band that transiently appears after wounding or bFGF treatment includes a core protein, cultures treated with bFGF were labeled for 6 h with \([^{35}S]\)methionine. After chondroitin ABC lyase treatment, SDS-PAGE revealed both the \([^{35}S]\)methionine-labeled \( M_r \)-sulfated GAG epitopes, respectively, or a chicken polyclonal antibody specific for biglycan (Ch105). Solid line and dashed line arrows mark intact biglycan core and biglycan N-terminal fragment positions, respectively.

Another distinct and transient change in the radiolabeled band pattern in PGs present in media from both wounded and bFGF-treated cultures is the appearance of a new band between the 43- and 68-kDa standard bands (Fig. 5, arrows). The low \( M_r \) band that is prominent in media samples is not apparent in cell layer extracts. To determine if the low \( M_r \) \([^{35}S]\)sulfate-labeled band that appears after wounding or bFGF treatment includes a core protein, cultures treated with bFGF were labeled for 6 h with \([^{35}S]\)methionine. After chondroitin ABC lyase digestion, SDS-PAGE revealed both the \([^{35}S]\)methionine-labeled \( M_r \)-o r4 -o sulfated GAG epitopes, respectively, or a chicken polyclonal antibody specific for biglycan (Ch105). Solid line and dashed line arrows mark intact biglycan core and biglycan N-terminal fragment positions, respectively.

In another experiment, PGs were partially purified by chromatography on DEAE-Sephacel from media of cultures treated for 12 h with bFGF (10 ng/ml). These isolates were then chondroitin ABC lyase-digested, run on SDS-PAGE, and Western blotted (Fig. 6B), using chicken anti-bovine biglycan (Ch105) or monoclonal antibodies (3B3 and 2B6) that recognize the terminal sulfated disaccharides that remain as “stubs” on the protein core after chondroitin lyase digestion. Both the anti-biglycan and the anti-stubs antibodies recognized bands on the Western blot of \( M_r \)-o r4 -o sulfated GAG epitopes, respectively, or a chicken polyclonal antibody specific for biglycan (Ch105). Solid line and dashed line arrows mark intact biglycan core and biglycan N-terminal fragment positions, respectively.

**Selective Expression of Biglycan**

![Image 352x581 to 520x729](http://www.jbc.org/)
Selective Expression of Biglycan

**Fig. 7.** Time course of incorporation of 35S-amino acids into biglycan core proteins. Secreted, chondroitin ABC-lyase-generated core proteins harvested at various times after culture manipulation from the culture medium of multiscratch wounded cultures after a 3-h labeling period (A) or bFGF-treated cultures after a 6-h labeling period (B) were separated by SDS-PAGE (see Fig. 6A). Intact biglycan (open bars) and N-terminal biglycan fragment (cross-hatched bars) were quantitated from fluorographs by scanning densitometry, as described under “Experimental Procedures,” and the values were normalized to moles of product. **Filled bars** represent the sum of intact and N-terminal fragment incorporation. Results are presented from single representative experiments.

thesis of longer chondroitin sulfate chains on both small and large PGs (22, 33). This observation raises the possibility that the described increase in [35S]sulfate incorporation into biglycan is primarily an effect on GAG chain synthesis. Therefore, the time course of biglycan core protein synthesis was quantitated after pulse-labeling with [35S]methionine at various times after multiscratch wounding or addition of 10 ng/ml bFGF to confluent cultures, to determine if biglycan core protein synthesis is increased (Fig. 7). Aliquots from bFGF-treated (Fig. 6A) or wounded cultures (not shown) run on SDS-PAGE showed the expected broad bands representing biglycan and its N-terminal fragment. Chondroitin ABC lyase digestion generates several core proteins including those of biglycan and biglycan fragment, at ~50 kDa and ~20 kDa, respectively. Digested cell layer aliquots contain less than 10% of the label present in comparable bands from media samples. Quantitation of radiolabel incorporated into total (intact + fragment) biglycan core indicates that biglycan core protein increases ~4.2-fold compared with untreated control cultures, with a maximal stimulation occurring between 6 and 12 h after bFGF addition (Fig. 7B). However, the largest component of increased core production is the N-terminal fragment, which accounts for about 85% of total biglycan core protein (on a molar basis) at the peak of bFGF-stimulated synthesis. A comparison of biglycan RNA expression after stimulation with bFGF or wounding (Fig. 9) indicates that biglycan RNA levels are elevated about 2-fold at 18 h after either treatment. However, expression of biglycan RNA remains elevated 24 h and 48 h after wounding, while in bFGF-treated cultures steady-state levels of biglycan mRNA decline at later time points. Biglycan mRNA expression appears to increase subsequent to both increased [35S]sulfate incorporation into biglycan GAG chains (see Figs. 2 and 5) and labeled methionine incorporation into biglycan core protein (see Figs. 6 and 7). This unexpected observation may suggest that early changes in biglycan core protein synthesis are not controlled at the level of mRNA transcription (see “Discussion”).

**Fig. 8.** Northern blots of the expression of PG mRNA in confluent and multiscratch wounded cultures. Total RNA isolated from confluent (unWo) cultures and multiscratch wounded cultures at 2, 5, 24, and 48 h after wounding were separated on a denaturing agarose gel (15 μg/lane), transferred to nitrocellulose, and probed with 32P-labeled cDNAs for the principal endothelial cell PGs, biglycan and perlecan, as well as versican, a large nonabundant chondroitin sulfate PG. A probe for 28 S ribosomal RNA was used to compare relative loading among the lanes. Autoradiographs were exposed at ~70 °C for 14 h (biglycan), 24 h (perlecan), 4 days (versican), and 12 h (28 S rRNA). Decorin expression (not shown), was barely detectable after a 9-day exposure.
small leucine-rich PGs, which contains at least 4 different proteins, including decorin. The members of this PG family are characterized by conserved cysteines that form disulfide-bonded loops near both termini of the protein core and highly homologous internal leucine-rich repeats, which comprise about 80% of the deduced sequences and have been postulated to mediate protein-protein interactions. Decorin (28) and biglycan (27) bear one or two dermanatan sulfate chains, respectively, at the N-terminal serine residues that support GAG chain substitution. Leucine-rich PGs, such as biglycan and decorin, may regulate cellular migration through several proposed mechanisms. As constituents of the extracellular matrix, the core proteins of decorin and biglycan are thought to interact with several different matrix proteins and influence matrix assembly. For example, the interaction of decorin with interstitial collagens affects collagen fibrillogenesis (8). Biglycan may interact with fibronectin (23) and types I (7) and VI (34) collagen. The matrix proteins with which leucine-rich PGs interact provide binding sites for adhesion receptors on cells within the extracellular matrix. The interaction of decorin and/or biglycan with fibronectin modulates cellular adhesion (10, 35), perhaps by influencing the binding of RGD-dependent cell surface receptors to sites on fibronectin. Such cooperative interactions have been proposed to destabilize focal adhesions, thereby modulating cellular migration (9). Also, biglycan and decorin bind to growth factors that may be important to vascular cell migration, including transforming growth factor-β (5, 36). This interaction may affect the availability or activity of these growth factors, which are important to the control of cell growth and migration (1, 2). Taken together, the hypotheses engendered by these observations suggest that induction of biglycan expression or cleavage during cell migration may be important to modulate either cell-substrate adhesion or influence the availability and function of growth factors with a demonstrated role in cell migration and angiogenesis. Interestingly, the expression of decorin is not up-regulated as macrovascular endothelial cells migrate after wounding, in contrast to published studies of PG expression by these cells during in vitro angiogenesis (16). This observation suggests that processes involved in the formation and stabilization of endothelial tubes during in vitro angiogenesis, which may require the induction of decorin expression, are clearly distinct from processes involved in cell movement, during which decorin expression is not induced.

Because bFGF is known to be released after wounding of endothelial cell monolayers (17), and modulates cell phenotype and behavior, the effects of exogenous bFGF on PG synthesis were compared to the modulation of PG synthesis as cells migrate after multiscratch wounding. In these experiments, addition of bFGF had similar effects on biglycan synthesis as wounding, including transient increases in RNA expression, core protein synthesis, size of the intact PG monomer, and biglycan turnover. Since a neutralizing antibody against bFGF inhibits the stimulation of [35S]sulfate incorporation into PG and proteolytic processing of biglycan in wounded or bFGF-treated cultures, we conclude that the release of endogenous bFGF is required for the modulation of these processes during cell migration. A notable difference in the effects of added exogenous bFGF and wounding on biglycan synthesis is that the release of the cells is delayed and persists for longer times in wounded cultures. This may suggest that release of bFGF from cell monolayers also persists for a relatively extended period of time after wounding.

In addition to increased sulfate incorporation into the GAG chains of biglycan, biglycan RNA expression and protein core synthesis are also elevated after bFGF treatment or wounding, indicating that the effect is not exclusive to stimulation of glycosyltransferase activity. However, the increased size of biglycan, which is attributable to the presence of longer GAG chains on the core protein (22, 33), is apparent earlier than either up-regulation of protein core synthesis or RNA expression. Therefore, GAG chain synthesis and protein core expression may be independently regulated by cells. In addition, the peak of stimulation of biglycan core protein synthesis clearly precedes the peak increases in mRNA expression for biglycan after either bFGF treatment or monolayer wounding (compare Figs. 7 and 9). This observation suggests that the control of biglycan synthesis by bFGF may be post-transcriptional, and that the subsequent smaller increase in biglycan transcript levels may be an indirect effect, perhaps due to the stimulation of an intermediate factor. Recent studies have indicated that, when transfected into the tumor cell line, MG-63, biglycan promoter constructs as well as the transcription from the endogenous biglycan gene are not up-regulated by TGF-β1 (39), despite an increase in biglycan RNA steady-state levels that is
induced by TGF-β1 treatment. These data are consistent with the regulation of biglycan expression through post-transcriptional mechanisms, at least under those conditions. It remains possible, however, that activation of TGF-β in EC cultures after wounding may be responsible for the delayed up-regulation of biglycan RNA expression. Although a few studies have indicated that bFGF increases the expression of some PGs (40, 41), cells may respond to other growth factors induced in wounded or bFGF-treated cultures. For example, transforming growth factor-β1, which is an antagonist of bFGF, activates gene transcription and increases synthesis and deposition of fibronectin, collagens, and the PGs, versican and biglycan (1, 22, 33, 41–43) and is activated by proteases induced by bFGF (44). In recent work, Ku and D’Amore (45) have demonstrated that the up-regulation of bFGF mRNA expression in response to release of bFGF following sublethal injury to endothelial cells can be abrogated by neutralizing anti-TGF-β1 antibodies or antiprotease strategies that reduce the activation of TGF-β. Because preliminary studies indicate that transforming growth factor-β1 also increases biglycan synthesis in endothelial cells, we cannot yet eliminate the possibility that such factors, activated or induced downstream from bFGF stimulation, are also involved in the control of biglycan metabolism.

Biglycan proteolytic processing, which results in the accumulation in the culture media of an N-terminal fragment of biglycan representing about one-half of the protein core, is dramatically stimulated in addition to increased biglycan synthesis by migrating or bFGF-treated cells. This observation raises several possibilities. First, if biglycan is required during the normal migration of cells after wounding, it may be that the functional portion of the molecule is one of the fragments that is generated, rather than the intact PG. Second, a migration-related cellular process may require the removal, rather than the increased production, of biglycan. While changes in biglycan expression and processing occurring during cell migration, it remains to be tested whether biglycan processing at the cell surface is required for the modulation of cell-adhesive interactions or the utilization of growth factors important to endothelial cell movement.

The rapid degradation of biglycan probably occurs at or near the cell surface, since intact biglycan released to the media is not degraded with time, and the N-terminal fragment accumulates in the culture supernatant, rather than in an intracellular compartment. Immunocytochemical staining indicates that cell-associated biglycan is distributed at the edges of lamellipodia of migrating cells. The localization of biglycan to these cellular structures is intriguing because these cell surface sites are critical both to cellular adhesive events and to the utilization of growth factors by cells, which are processes in which leucine-rich PGs may play a role. Urokinase plasminogen activator, which generates active plasmin from plasminogen, has been localized to focal adhesions (46), which are concentrated on cell processes. Both metalloproteinases and serine proteases, such as urokinase-plasminogen activators, are strongly induced in endothelial cells by bFGF (47), which promotes cell migration and angiogenesis (2). The induction of such proteases at the cell surface occurs concurrent to cell migration (1, 2, 48), although specific substrates are not fully known. However, substrates include matrix proteins, such as collagens (49), as well as cell surface-associated biglycan. Preliminary studies indicate that the serine protease inhibitor, aprotinin, inhibits the cleavage of biglycan in FGF-stimulated endothelial cell cultures, and that plasmin generates cleavage products from purified calf cartilage biglycan similar to those that are found in stimulated endothelial cell cultures, including a ~20-kDa N-terminal fragment. These observations raise the possibility that plasmin, which is activated by urokinase-induced bFGF, may be responsible for biglycan processing during cell migration.

In summary, endothelial cell migration is controlled by a complex series of interactions between cells and both soluble growth regulatory molecules and the extracellular matrix. Evidence has accumulated that leucine-rich PGs, such as decorin and biglycan, are involved in the modulation of cellular adhesion and growth factor activity, and biglycan is present at cell surface sites that suggest a role in those processes. Clearly, biglycan expression and turnover are rapidly and transiently modulated as cells migrate following wounding. In addition, the alteration of biglycan metabolism after monolayer wounding is controlled by release of endogenous bFGF, which is a principal factor that regulates endothelial cell proliferation, migration, and differentiation (2). However, whether changes in biglycan expression or turnover are required for normal cell migration or the maintenance of a differentiated phenotype remain to be tested.

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REFERENCES

1. Roberts, A. B., and Sporn, M. B. (1989) Am. Rev. Respir. Dis. 140, 1126–1128
2. Folkman, J., and Klagsbrun, M. (1987) Science 235, 442–447
3. Hynes, R. O. (1987) Cell 54, 529–544
4. Wight, T. N. (1989) Arteriosclerosis 9, 1–20
5. Russel, T. E., and Yamaguchi, Y. (1991) Cell 64, 867–869
6. Scott, J. E., and Orford, C. R. (1981) Biochem. J. 197, 213–216
7. Schönherr, E., Witsch-Premh, R., Harrach, B., Robenek, H., Rauterberg, J., and Kresse, H. (1995) J. Biol. Chem. 270, 2776–2783
8. Vogel, K. G., Paulsson, M., and Heinégard, D. (1984) Biochem. J. 223, 587–597
9. Couchman, J. R., Austria, M. R., and Woods, A. (1991) J. Invest. Dermatol. 94, 75–145
10. Bidanset, D. J., LeBaron, R., Rosenberg, L., Murphy-Ullrich, J. E., and Hoek, M. (1992) J. Cell Biol. 118, 1523–1531
11. Kinsella, M. G., and Wight, T. N. (1986) J. Cell Biol. 102, 679–687
12. Kinsella, M. G., and Wight, T. N. (1988) J. Biol. Chem. 263, 19222–19231
13. Jarvelainen, H. T., Kinsella, M. G., Wight, T. N., and Sandell, L. J. (1991) J. Biol. Chem. 266, 23274–23281
14. Voss, B., Gijs, J., Cully, Z., and Kresse, H. (1986) J. Histochem. Cytochem. 34, 1019–1029
15. Bianco, P., Fisher, L. W., Yousef, M. F., Termine, J. D., and Robey, P. G. (1990) J. Histochem. Cytochem. 38, 1549–1563
16. 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
17. McNeil, P., Muthukrishnan, L., Warder, E., and D’Amore, P. A. (1989) J. Cell Biol. 109, 811–822
18. Lindner, V., Lappi, D. A., Baird, A., Majack, R. A., and Reidy, M. A. (1991) Circ. Res. 68, 106–113
19. Wasteson, Å., Uthe, K., and Westermark, B. (1973) Biochem. J. 136, 1069–1074
20. Saito, H., Yamagata, T., and Suzuki, S. (1968) J. Biol. Chem. 243, 1536–1542
21. Laemmli, U. K. (1970) Nature 227, 680–685
22. Schönherr, E., Jarvelainen, H. T., Kinsella, M. G., Sandell, L. J., Sage, E. H., and Wight, T. N. (1990) Arterioscler. Thromb. 10, 1026–1036
23. Kinsella, M. G., and Wight, T. N. (1990) J. Biol. Chem. 265, 17891–17898
24. Bjerrum, O. J., and Schafer-Nielsen, C. (1986) Biochem. J. 236, 679–687
25. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
26. Fisher, L. W., Termine, J. D., and Young, M. F. (1989) J. Biol. Chem. 264, 4571–4576
27. Doy, A. A., McQuillan, C. I., Termine, J. D., and Young, M. R. (1987) Biochem. J. 248, 801–805
28. McQuillan, C. I., and Doy, A. A. (1990) J. Cell Biol. 116, 17891–17898
29. Groud, D. C., Dodge, G. R., Cohen, I., Tuan, R. S., and Iozzo, R. V. (1992) J. Biol. Chem. 267, 8454–8457
30. Kremnitz, T., Gehlsen, K. R., and Russel, H. E. (1987) J. Biol. Chem. 262, 13120–13125
31. Saku, T., and Furmey, H. (1989) J. Biol. Chem. 264, 3514–3523
32. Klebanoff, S. J., Kinsella, M. G., and Wight, T. N. (1990) Am. J. Pathol. 140, 907–917
33. Bassol, A., and Massagut, J. (1988) J. Biol. Chem. 263, 3039–3045
34. Bidanset, D. J., Guiraud, C., Rosenberg, L. C., Choi, H. U., Timpl, R., and Hook, M. (1992) J. Biol. Chem. 267, 5250–5256
35. Winnemoller, M., Schon, P., Vischer, P., and Kresse, H. (1992) Eur. J. Cell Biol. 59, 47–55
36. Yamaguchi, Y., Mann, D. M., and Russelh, E. (1990) Nature 346, 281–284

3 M. G. Kinsella, personal observation.
37. Deleted in proof
38. Deleted in proof
39. Ungefroren, H., and Krull, N. B. (1996) J. Biol. Chem. 271, 15787–15795
40. Tan, E. M. L., Dodge, G. R., Sorger, T., Kovalsky, I., Unger, G. A., Yang, L., Levine, E. M., and Iozzo, R. V. (1991) Lab. Invest. 64, 474–482
41. Elenius, K., Maatta, A., Salmivirta, M., and Jalkanen, M. (1992) J. Biol. Chem. 267, 6435–6441
42. Schönherr, E., Jarvelainen, H. T., Sandell, L. J., and Wight, T. N. (1991) J. Biol. Chem. 266, 17640–17647
43. Ignotz, R. A., Endo, T., and Massagué, J. (1987) J. Biol. Chem. 262, 6443–6446
44. Sato, Y., and Rifkin, D. B. (1989) J. Cell Biol. 109, 309–315
45. Ku, P.-T., and D’Amore, P. A. (1995) J. Cell. Biochem. 58, 328–343
46. Pullanen, J., Hedman, K., Nielsen, L. S., Dano, K., and Vahari, A. (1988) J. Cell Biol. 106, 87–95
47. Gross, J. L., Moscatelli, D., Jaffe, E. A., and Rifkin, D. B. (1982) J. Cell Biol. 95, 974–981
48. McGuire, P. G., and Alexander, S. M. (1993) Development 118, 931–939
49. Woessner, J. F., Jr. (1991) FASEB J. 5, 2145–2154
Selective Expression and Processing of Biglycan during Migration of Bovine Aortic Endothelial Cells: THE ROLE OF ENDOGENOUS BASIC FIBROBLAST GROWTH FACTOR

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