Regulation of Basic Fibroblast Growth Factor Binding and Activity by Cell Density and Heparan Sulfate*

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Thomas P. Richardson, Vickery Trinkaus-Randall, and Matthew A. Nugent‡
From the Departments of Biochemistry and Ophthalmology, Boston University School of Medicine, Boston, Massachusetts 02118

The role of cell density in modulating basic fibroblast growth factor binding and activity was investigated. A primary corneal stromal fibroblast cell culture system was used, since these cells do not constitutively express heparan sulfate proteoglycans in vivo except after injury. A 3–5-fold reduction in bFGF binding per cell was observed as cell density increased from 1000 to 35,000 cells/cm². The cell density-dependent change in bFGF binding was not the result of altered FGFR expression as determined by equilibrium binding experiments and by immunoblot analysis. However, bFGF-cell surface receptor binding affinities were measured to be 10–20-fold higher at low cell densities than at intermediate and high cell density. bFGF-induced cell proliferation was also cell density-dependent, with maximal stimulation of proliferation 190–280% greater at intermediate densities (15,000 cells/cm²) than at other cell densities. This effect was specific to bFGF as serum, epidermal growth factor, and transforming growth factor-β did not exhibit the same density-dependent profile. Further, heparan sulfate proteoglycans and, specifically, syndecan-4 were implicated as the modulator of bFGF binding and activity. Pretreatment of cell cultures with heparinase resulted in reduced bFGF binding to the cells and abrogated bFGF induced proliferation. These data suggest a mechanism by which cell density regulates heparan sulfate proteoglycan expression and modulates the cellular response to bFGF. Modulation of heparan sulfate proteoglycan expression might be an important aspect of the regulation of stromal cell migration and proliferation during wound healing.

Basic fibroblast growth factor (bFGF) mediates many biological processes including cell proliferation, differentiation, angiogenesis, and wound healing (1). It is a member of a family of at least 12 heparin-binding proteins. The activity of bFGF is mediated by binding to heparan sulfate proteoglycans (HSPG) and to high affinity, cell surface receptor tyrosine kinases (FGFR).

bFGF can be regulated by HSPG, which serve as low affinity receptors for bFGF (2). The role of HSPG in modulating bFGF activity has been described at many levels (2–8). In their role as a co-receptor for bFGF, HSPG participate in a ternary complex with bFGF and FGFRs (9–12). This complex has high affinity for bFGF, with Kd values ranging from 10^-10 to 10^-12 M. The high affinity binding of bFGF has been attributed to a slow dissociation of bFGF from the ternary complex (9, 13). The generation of stable, high affinity bFGF:FGFR complexes is probably a major mechanism leading to HSPG-dependent bFGF activity. In addition, bFGF has been localized to the extracellular matrix, associated with HSPG (6, 14, 15). Release of bFGF by matrix degradation may serve as a mechanism for mobilizing bFGF in response to injury or tissue reorganization (16, 17).

Studies using HSPG-deficient cells have demonstrated that bFGF can bind FGFR and induce activity in the absence of HSPG, yet HSPG appear necessary for physiologic, bFGF-induced cellular response (3, 4, 5, 11). HSPG do not always lead to potentiation of bFGF. Endothelial cell-derived HSPG have been demonstrated to have an inhibitory effect on smooth muscle cell proliferation, indicating that HSPG are able to sequester bFGF from the cell surface (6). Furthermore, HSPG have an emerging role in the intracellular processing of bFGF in which HSPG are thought to stabilize bFGF through endosomal and lysosomal trafficking (8).

Since HSPG can influence bFGF binding and activity, much research has focused on the regulation of HSPG as a major control mechanism for bFGF. Studies on differentiating neural cells have demonstrated changes in binding affinity of HSPG for bFGF, attributable to differential HSPG expression (18). Further, studies in vivo have demonstrated localized HSPG expression at the leading edge of healing corneal wounds (19). The corneal stroma has a highly defined tissue architecture, where cells make contact via gap junctions between collagen lamellae (20). The stromal cells do not constitutively express HSPG. This suggests a role for intercellular contact as a regulatory mechanism for differential HSPG expression where cells sense the environment via contact with adjacent cells and matrix components. Hence, intracellular signaling mediated by cell density changes could potentially modulate HSPG expression. Since the cornea is both avascular and devoid of direct lymphatic supply and thus elicits the wound response without significant, direct influence of blood and lymph, it is hypothesized that the corneal stroma is very sensitive to localized changes in intercellular contact and extracellular matrix organization. These changes might signal the cells at the wound edge to express HSPG and provide stromal fibroblasts with a mechanism for bFGF modulation.

In the present study, the regulation of bFGF binding to the cell surface of stromal fibroblasts was analyzed and correlated to changes in bFGF-induced proliferation. Cells were cultured at various cell densities to model changes in intercellular con-
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Binding of 125I-bFGF—Preparation of 125I-bFGF with 125I-Bolton-Hunter reagent was conducted as described by Nugent and Edelman (9). Equilibrium binding was measured on cultures at 4 °C in binding buffer (DMEM, low glucose, with 25 mM HEPES, 0.05% gelatin) (5, 9). Cultures were incubated for 2.5 h at 4 °C with 125I-bFGF and rinsed with ice-cold binding buffer three times. bFGF bound to HSPG was released with rapid washes of a high salt buffer (2 M NaCl, 20 mM HEPES, pH 7.4) and phosphate-buffered saline (PBS). Receptor-bound bFGF was released with a 10-min wash with a high salt, low pH buffer (2 M NaCl, 20 mM NaCH3COOH, pH 4.0), followed by a quick rinse with PBS. Bound 125I-bFGF was quantified by counting in an Auto-Gamma -counter (Packard Instruments, Downer’s Grove, IL). Nonspecific binding of 125I-bFGF was measured by adding unlabeled bFGF (278 nM) prior to the addition of 125I-bFGF. To determine the effects of removing specific glycosaminoglycans on bFGF binding, cells were treated with specific glycosaminoglycan-degrading enzymes prior to the binding studies. Cells were treated with heparinase I (1.5 μg/ml), chondroitinase ABC (0.1 units/ml), or keratanase/endo-β-galactosidase (0.1 unit/ml). The enzymes and glycosaminoglycan digestion products were removed by washing with binding buffer, and 125I-bFGF binding was measured. Identification of FGFR1 by Immunoblot—To determine the relative expression of FGFR on various cell densities, cells were plated at various cell densities as described and incubated at 37 °C for 24 h. Cultures were homogenized by scraping in extraction buffer (20 mM HEPES, 1% Triton X-100, 10% glycerol, 2 mM phenylmethylsulfonyl fluoride), and nuclei and insoluble debris were removed by centrifugation at 12,000 × g for 10 min (5). Cell extracts were subjected to SDS-PAGE (7%) under reducing conditions, and the proteins were transferred to Immobilon-P membrane (Millipore Corp., Bedford, MA). The membrane was blocked with PBS (1% Tween 20), 5% nonfat milk for 1 h at room temperature, and the antibody to FGFR1 (1:1000) was incubated with the membrane for 1 h at 37 °C. After rinsing three times with PBS (0.1% Tween 20), the membrane was incubated with anti-mouse IgG-horseradish peroxidase (1:5,000) secondary antibody and rinsed three times with PBS, 1% Tween 20. Visualization was conducted by enhanced chemiluminescence using Renaissance reagents from NEN Life Science Products. Determination of FGFR Identity by Cross-linking—To further identify FGFR, bFGF-FGFR complexes were covalently cross-linked by disuccinimidyl suberate (5). FGFR and bFGF were identified by immunoblots. Cells were plated at high density and cultured for 24 h as described. The bFGF binding assay was performed as described above, using unlabeled bFGF. Then cell layers were washed three times with PBS. Fresh PBS (5 ml) was then added to cultures, and disuccinimidyl suberate (300 μl) was added to cross-link bFGF-FGFR complexes. Culture dishes were gently shaken for 20 min at room temperature, and the reaction was quenched by adding 500 μl of buffer (500 mM Tris-HCl, pH 8.0, 1.0 mM glycyne). Cells were rinsed three times with PBS and homogenized by scraping in extraction buffer (20 mM HEPES, 1% Triton X-100, 10% glycerol, 2 mM phenylmethylsulfonyl fluoride). Nuclei and insoluble debris were removed by centrifugation at 12,000 × g for 10 min. Cell extracts were subjected to SDS-PAGE (7%), proteins were transferred to Immobilon-P membrane, and the membrane was blocked with PBS (1% Tween 20), 5% nonfat milk for 1 h at room temperature. For FGFR analysis, immunoblots were performed as described. For bFGF visualization, blots were hybridized with anti-bFGF (1:1000) using the procedure described for FGFR. Density Dependence of Mitogen-stimulated Proliferation—Mitogen activity was assessed by determining the relative amount of acid phosphatase activity present in each culture after incubation with various mitogens (23). The level of acid phosphatase has previously been demonstrated to relate directly to cell number in a variety of systems. We used specific acid phosphatase levels to monitor cell number in our studies to ensure that all cultures were treated identically. Acid phosphatase levels showed a linear relationship with corneal fibroblast cell number under our culture conditions. Cells were plated at several cell densities and incubated for 24 h, as described. Cultures (t = 24) were treated with PBS, bFGF (0.56 nm), TGF-β (0.4 nm), epidermal growth factor (1 nm), or PBS (to a total PBS concentration of 20%) and incubated for 24 h (t = 48). Cell number in replicate cultures was determined by a Coulter cell counter.
counter. One plate was rinsed with PBS and placed at −20 °C to serve as the initial cell density condition for acid phosphatase activity determination at t = 24. At t = 48, cells were rinsed with PBS and placed at −20 °C for 2 h. The amount of cellular acid phosphatase activity was quantified in each culture to determine relative cell numbers. Cells were lysed with buffer (0.1% Triton X-100, 100 mM NaCl, 1 mM CH$_3$COO, pH 5.5) and assayed for acid phosphatase activity upon the addition of para-nitrophenol phosphate. The role of HSPG in regulating proliferation was determined by treating cultures at cell density of 14,000 cells/cm$^2$ with 1.0 units/ml heparinase III for 1 h prior to the addition of bFGF.

**Determination of Syndecan-4 by Immunoblot**—Cells were cultured for 24 h at various cell densities as described above. Cells were digested with heparinase III, extracted, and subjected to SDS-PAGE. After transfer of proteins to polyvinylidene difluoride, the membrane was blocked with PBS (0.1% Tween 20), 5% nonfat milk overnight and hybridized with anti-syndecan-4 antibody (1:500) for 1 h at 37 °C. The membrane was then washed three times with PBS (0.1% Tween 20) and hybridized with an anti-mouse IgG-horseradish peroxidase secondary antibody (1:5,000) for 1 h at 37 °C. Syndecan-4 was visualized as described above.

**RESULTS**

**Cell Density Dependence of bFGF-stimulated Proliferation**—The corneal stroma has a defined architecture of cells, collagen lamellae, and associated proteoglycans. The cells comprising this tissue communicate via gap junctions (20, 24). To investigate the role that cell-cell communication plays in modulating the mitogenic response of corneal fibroblasts, growth factor-induced cell proliferation was analyzed in cells at different densities. To determine if responsiveness was dependent on initial plating density or on cell density in general, we examined mitogen-induced cell proliferation in cultures plated at specific cell densities and in cultures plated at a single low density and then grown to various higher densities. To conduct the experiments, cells were cultured at a particular density for 24 h, thereby establishing density-dependent levels of intercellular contact. Then the cultures were treated with epidermal growth factor, TGF-β, bFGF, or serum. From replicate cultures, cell number was determined with a Coulter counter to yield the exact cell density at the time of growth factor incubation. Cell proliferation was assayed by determining relative levels of acid phosphatase activity. Specifically, the percentage of proliferation was determined by measuring acid phosphatase activity at the time of mitogen administration and again after 24 h and comparing the changes to those observed for untreated controls. bFGF stimulated cell proliferation at all cell densities tested (Fig. 1). However, at cell densities between 12,000 and 16,000 cells/cm$^2$, bFGF-stimulated cell proliferation was dramatically increased (190–280%) compared with that observed at other cell densities (40–100%). This effect was experimentally determined five times. Epidermal growth factor, TGF-β, bFGF, or serum. From replicate cultures, cell number was determined with a Coulter counter to yield the exact cell density at the time of growth factor incubation. 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bFGF Binding Decreases as Cells Proliferate—Since mitogenic responsiveness to bFGF varied with cell density, it was possible that the expression of cell surface binding sites also varied. Thus, we investigated the role of cell density as a mechanism by which bFGF binding to the cell surface can be modulated. As cells grew from low density (2000 cells/cm²) to high density (19,000 cells/cm²) over 96 h (Fig. 2A), we observed a 3–5-fold decrease in binding of bFGF per cell (Fig. 2B). Cells in low density cultures (500–3000 cells/cm²) bound 0.5–1.4 × 10⁶ molecules of bFGF/cell, whereas cells at higher densities (20,000–35,000 cells/cm²) bound 150,000–250,000 molecules/cell. bFGF binding to cells plated at various cell densities and cultured for 24 h exhibited a similar 3–5-fold increase in bFGF binding per cell at low cell density compared with high cell density (Fig. 2C).

bFGF Binding to FGFR1 and HSPG—Both FGFR and HSPG can independently bind bFGF and, can act together to form a high affinity, ternary complex (7, 9, 10). We sought to determine the role of each in binding bFGF by measuring the relative amount of bFGF bound to each class of sites. The amount of bFGF bound to these two classes of binding sites can be measured by using selective extraction conditions to remove HSPG-bound bFGF from FGFR-bound bFGF (2). Binding of bFGF was measured at bFGF concentrations of 27.8 pM to 1.11 nM in cell cultures at densities of 4000, 15,800, and 25,900 cells/cm². At the highest concentration of bFGF tested (1.11 nM), cells, at densities of 4000, 15,800, and 25,900 cells/cm², bound a total of 13.3 × 10⁶, 7.19 × 10⁵, and 5.11 × 10⁵ molecules of bFGF/cell, respectively (Fig. 3A). bFGF bound to HSPG sites at the same densities was measured to be 13.0 × 10⁵, 6.70 × 10⁵, and 4.46 × 10⁵ molecules/cell, respectively (Fig. 3B). K_d values for HSPG interactions were calculated to be 7.8 ± 6.7 and 7.8 ± 7.8 nM for cell densities of 4000 and 15,800 cells/cm², respectively. The receptor-bFGF interactions were calculated to have K_d values of 23 ± 15, 200 ± 77, and 384 ± 104 pm for cell densities of 4000, 15,800, and 25,900 cells/cm², respectively. Receptor number/cell was calculated to be (6.9 ± 0.7) × 10⁴, (6.3 ± 0.8) × 10⁴, and (10.0 ± 1.2) × 10⁴ molecules/cell.

Receptor Expression Does Not Vary with Cell Density—The binding data suggested that the levels of FGFR1 expression did not vary significantly. Further, differences in the K_d for bFGF binding at different cell densities appeared to result from HSPG. To further evaluate the changes in bFGF binding to receptor, immunoblots were performed to compare relative levels of FGFR1 expression at different cell densities using an antibody raised against the extracellular domain of FGFR1. One prominent 73-kDa species was detected, and the expression level did not appear to vary significantly with cell density (Fig. 4A). This band was also detected using a second monoclonal antibody (Upstate Biotechnology; data not shown).

Determination of 73-kDa FGFR1—Although the immunore-
active 73-kDa band does not directly correspond to any described isoforms of FGFR1. FGFR isoforms have previously been shown to be subject to differential expression and processing yielding a range of molecular masses of 55–145 kDa (25–28). To further identify the 73-kDa species as an FGFR, we performed immunoblots for bFGF cross-linked to its receptor and visualized both bFGF and FGFR1 bands by enhanced chemiluminescence. To one half of the membrane, an anti-bFGF monoclonal antibody was hybridized and visualized. This detected a band at 91 kDa, corresponding to the combined molecular masses of bFGF (18 kDa) and the 73-kDa species (Fig. 4B). Also detected were bands at 18 and 36 kDa, corresponding to bFGF and bFGF dimers, respectively. Cross-linked bFGF to FGFR1 was also detected in samples not treated with bFGF, indicating the presence of endogenous bFGF. To the other half of the membrane, an anti-FGFR1 monoclonal antibody was hybridized and visualized. This detected a 73-kDa species and a 91-kDa species only when cells were treated with disuccinimidyl suberate (Fig. 4B). Control blots hybridized only to secondary antibody did not show any bands (not shown).

**Heparinase Treatment Abrogates bFGF Binding and bFGF-stimulated Proliferation**—The changes in bFGF binding versus cell density were not mediated by different isoforms of FGFR1 expression. From the binding data, HSPG appeared to be the major regulatory mechanism by which these cells modulate bFGF binding. To test this hypothesis, cell cultures at various cell densities were digested with enzymes to degrade specific classes of glycosaminoglycans. Binding was measured and compared with untreated cell cultures. At a cell density of 15,300 cells/cm², digestion with chondroitinase ABC and keratanase II/endo-β-galactosidase resulted in bFGF binding levels relative to control of 94 and 99%, respectively (Fig. 5). Heparinase I digestion resulted in complete loss of specifically bound bFGF. Similar results were obtained for the cell density of 30,600 cells/cm². Interestingly, at low cell density (3000 cells/cm²), chondroitinase ABC and keratanase II/endo-β-galactosidase digestion resulted in levels of bFGF binding relative to control of 59 and 80%, respectively. Heparinase I completely abrogated specific binding of bFGF to these low density cultures, as in higher density conditions. To correlate bFGF binding interactions to the density-dependent proliferation profile induced by bFGF (Fig. 1), we treated cultures within the critical density range of 10,000–20,000 cells/cm² with heparinase III prior to stimulation with bFGF. The percentage of proliferation was determined by measuring the changes in acid phosphatase activity at the time of enzyme digestion to those 24 h later and comparing the changes to those observed for undigested controls. Treatment with bFGF resulted in a 139% stimulation of proliferation. Cultures digested with heparinase III and then treated with bFGF resulted in only 8% stimulation of proliferation. Digestion with heparinase III alone resulted in 22% less proliferation than untreated controls, further indicating the presence of endogenous bFGF (Fig. 6).

**Syndecan-4 Expression Decreases at Higher Cell Densities**—Differential HSPG expression was predicted to be the regulatory mechanism by which these cells change their binding capacity for bFGF. Since syndecan-4 has been demonstrated to be a major cell surface HSPG (29), its expression was measured...
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Fig. 6. Abrogation of bFGF-induced cell proliferation by heparinase pretreatment. Cells were plated and cultured 24 h. Cell number was determined with a Coulter counter to yield cell density at time of heparinase III/bFGF treatment. Replicate cultures at a cell density of 14,000 cells/cm² were treated with heparinase III (1.0 unit/ml) for 1 h at 37 °C. To heparinase III-treated or -untreated control cultures, bFGF (0.56 nM) was added, and cultures were incubated for 24 h. Stimulation of proliferation is represented as described above. Results represent the average of triplicate determinations. S.E. values are given and are not visible if smaller than the symbol.

DISCUSSION

The importance of co-receptors, like HSPG for bFGF binding, has been widely studied (2, 3, 9, 30–32), yielding significant insight into the mechanism and regulation of growth factor activity. Interestingly, many tissues contain high levels of several growth factors yet do not appear to be constitutively responding to their stimuli. For example, the aqueous humor has been reported to contain high concentrations of bFGF, yet the corneal endothelial cells and keratocytes appear quiescent in situ (33). These observations suggest that cellular response might be regulated by the competency of the cells and not solely by the presence of growth factors and their effectors. In this study, we investigated the action of bFGF in primary corneal stromal fibroblast cells cultured at different cell densities. We hypothesized that cell density serves as a mechanism by which cells sense their extracellular environment and control their response to growth factors. We found that bFGF binding was inversely proportional to cell density. Furthermore, we observed that bFGF-stimulated cell proliferation was also density-dependent. Thus, cell density might coordinate extracellular stimuli with cellular context to provide a concerted cellular response.

As cell density increased with proliferation, binding of bFGF per cell decreased 3–5-fold. This effect was not simply a result of ligand depletion at high densities, since analysis of the binding data revealed ligand depletion of less than 20%, except at very low concentrations of bFGF (27.8 pm). The binding data further indicated that cells plated at various cell densities and cultured for 24 h, as compared with those proliferating to specific densities, exhibited the same density-dependent changes in bFGF binding. These data indicate that cell density, whether achieved by plating conditions or by culturing to specific densities, provides the cells a mechanism by which bFGF binding may be modulated. Our data emphasize a role for HSPG as the primary means by which these cells modulate bFGF binding. However, it is important to note that the changes we observed might not be a direct response to cell density per se but instead could reflect the action of additional factors present in our cell culture system.

Experiments measuring bFGF-FGFR binding suggested that receptor number did not change with cell density. Indeed, immunoblot analysis showed no density-dependent changes in FGFR1. In contrast, receptor affinity was increased at lower cell densities. Determination of FGFR dissociation constants revealed a 10–20-fold higher affinity at the lowest cell density compared with the highest cell density (23 pm versus 200–384 pm). Thus, receptor affinity appeared to increase with increased expression of HSPG sites, consistent with a cooperative role for HSPG in stabilizing bFGF-FGFR complexes. We also have investigated HSPG core protein expression and identified syndecan-4 as an HSPG species regulated by cell density.

Our data indicated a 73-kDa species as a bFGF binding protein in our culture system. This molecular mass does not correspond to any known isoform of FGFR1, although several low molecular mass receptor species have been described (25–27). Due to the size, we speculate that this protein represents a previously unknown or processed form of FGFR1 or perhaps a proteolytically cleaved ectodomain of FGFR1 liberated by our extraction methods. The epitope of one of the monoclonal antibodies used to detect FGFR was generated using the ectodomain of FGFR1 and that of the other has not been determined. We therefore cannot formally conclude whether this 73-kDa protein is a fragment or a full-length receptor species. We also detected the presence of endogenous bFGF. While not directly measured, evidence that endogenous bFGF exists is 2-fold. First, cells treated for cross-linking in the absence of exogenously added bFGF exhibited a 91-kDa band corresponding to the sum of the 73-kDa FGFR and the 18-kDa bFGF. Second, pretreatment of cell cultures with heparinase I resulted in

Fig. 7. Expression of syndecan-4 decreases at higher cell density. Cells were plated and cultured 24 h. Cell number was determined with a Coulter counter to yield cell density at the time of heparinase digestion. Cells were extracted and digested with heparinase III as described under “Experimental Procedures.” Samples were subjected to SDS-PAGE, transferred to a polyvinylidene difluoride membrane, hybridized with a monoclonal antibody to syndecan-4, and visualized by ECL. Cell densities were determined to be 2, 10, and 25 × 10³ cells/cm² in lanes 2, 3, and 4, respectively. As a control, heparinase III alone was loaded in lane 1.
lower levels of basal cell proliferation compared with untreated controls, indicating a loss of sensitivity to endogenous bFGF-induced proliferation.

Changes in cell phenotype resulting from different levels of cell-cell contact have been shown to modulate HSPG expression (18, 34–37). In studies on proliferating smooth muscle cells, HSPG expression was determined to depend on cell phenotype (38). Specifically, HSPG from cells at high density had longer glycosaminoglycan chains than those at lower cell density. HSPG derived from nondividing smooth muscle cells had 10-fold higher antiproliferative potency than HSPG from proliferating cells. Other investigators have measured changes in affinity and binding of bFGF in differentiating neural cells and have determined that the changes described were due to modulation of HSPG expression (18). Together, these studies strongly correlate cell phenotype with differential HSPG expression.

Biochemical analyses of corneal wounds in vivo have demonstrated changes in proteoglycan synthesis, in which heparan sulfate and chondroitin sulfate/dermatan sulfate expression increase and keratan sulfate decreases (19, 39, 40). Alternatively, in situ hybridization has been successfully employed to detect HSPG at the leading edge of wounds. In vitro studies of wound healing have largely utilized linear wounds of confluent cell layers. These studies have shown somewhat similar data as in vivo, although they have been limited by the sensitivity of the assays used to describe the biochemical of the wound healing process. It was our aim to model intercellular communication levels that might be associated with wound healing and development. It has been previously demonstrated that functional gap junctions and focal adhesions form in these cells (20, 24), indicating a putative sensing mechanism of cell density. Further, in corneal fibroblasts cultured at very low cell density, a phenotypic change has been demonstrated, whereby a population of fibroblasts become myofibroblasts, exhibited by detection of α-smooth muscle actin (41). This phenotypic change would presumably allow for motility and, eventually, wound contraction. Indeed, changes in motility induced by fibroblast growth factor have been demonstrated in cells at low cell density, while the same growth factor induces mitogenesis at higher cell density (42). The mechanism of this conversion in cellular response was not resolved. Our data show markedly increased bFGF binding in cells at low cell density, potentially indicating a sequestration and protection of bFGF. The implications of this are not yet clear. It is possible that bFGF could induce cell migration until a critical cell density is achieved, whereby the mitogenic effects of bFGF would be dominant and cell proliferation would be stimulated. Thus, the maximal binding observed at low cell density did not correlate to maximal mitogenic potential. Only at intermediate densities were the mitogenic effects of bFGF maximal. Both the mitogenic effects and the binding effects were HSPG-mediated as demonstrated by the abrogation of bFGF-induced proliferation and of bFGF binding after heparinase treatment on the cell cultures.

Our data indicate that changes in bFGF binding and activity at different cell densities are HSPG-mediated. HSPG, by serving as low affinity, high capacity binding sites for bFGF, are required for the unique functions of this and possibly other heparin-binding growth factors (7). By protecting and sequestering bFGF, HSPG may serve to provide a developing or healing tissue with a constant source of bFGF, with the cellular response being dictated by numerous stimuli. HSPG expression could function to provide spatial and temporal regulation of bFGF and other heparin-binding growth factors to coordinate biological processes.

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