Heparin-binding EGF-like Growth Factor Stimulation of Smooth Muscle Cell Migration: Dependence on Interactions with Cell Surface Heparan Sulfate

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Abstract. Heparin-binding EGF-like growth factor (HB-EGF), but not EGF, binds to cell surface heparan sulfate proteoglycan (HSPG). This was demonstrated in (a) the binding of 125I-HB-EGF to mutant CHO cells deficient in HS production was diminished by 70% compared to wild-type CHO cells, (b) the binding of 125I-HB-EGF to CHO cells and bovine aortic smooth muscle cells (BASMC) was diminished 80% by heparitinase or chlorate treatment, and (c) 125I-EGF did not bind to CHO cells and its binding to BASMC was not diminished at all by heparitinase and only slightly by chlorate treatment. Accordingly, the role of HB-EGF interactions with HSPG in modulating bioactivity was examined. Heparitinase or chlorate treatment of BASMC diminished the ability of HB-EGF to stimulate BASMC migration by 60–80%. A similar inhibition of migration occurred when BASMC were treated with a synthetic peptide (P21) corresponding to the sequence of the putative heparin-binding domain of HB-EGF. As a control for BASMC viability, and for specificity, it was found that heparitinase and P21 did not inhibit at all and chlorate inhibited only slightly the stimulation of BASMC migration by PDGF AB. Since heparitinase, chlorate, and P21 treatment also diminished by 70–80% the cross-linking of 125I-HB-EGF to the EGF receptor, it was concluded that the interaction of HB-EGF, via its heparin-binding domain, with cell surface HSPG was essential for its optimal binding to the EGF receptor on BASMC and hence for its optimal ability to stimulate migration.

Heparin-binding EGF-like growth factor (HB-EGF) is a recently described member of the EGF family that was first identified as a secreted product of macrophages and macrophage-like cells (1, 2, 9, 10). Subsequently, HB-EGF has been found to be expressed by vascular endothelial cells (28), vascular smooth muscle cells (5, 5a), and to be a component of wound fluid (17). HB-EGF, which has been found to contain up to 86 amino acids (10) is significantly longer than EGF and the structurally homologous mitogen, TGF-α, which have sizes of 53 and 50 amino acids, respectively (4). The COOH-terminal region of HB-EGF contains six cysteine residues with the spacing characteristic of members of the EGF family (4) and shares ~40% sequence identity with EGF and TGF-α. Comparative structural examination suggests that HB-EGF is analogous to an NH₂-terminally extended form of EGF and TGF-α. Since EGF and TGF-α do not bind to heparin (2, 13) while HB-EGF binds tightly to heparin affinity columns and is eluted with 1–1.2 M NaCl (2, 9, 10), it appeared likely that the heparin-binding domain of HB-EGF would be somewhere upstream of the COOH-terminal EGF, TGF-α-like domain. Site-directed mutagenesis of HB-EGF and studies with peptide fragments have indicated that the heparin-binding sequences of HB-EGF reside primarily in a twenty one-amino acid stretch upstream of and slightly overlapping the EGF-like domain (Thompson, S., S. Higashiyama, K. Wood, M. Klagsbrun, and J. Abraham, manuscript in preparation).

HB-EGF is as active as PDGF but considerably more potent than EGF or TGF-α in stimulating bovine aortic smooth muscle cells (BASMC) proliferation (9) and migration (as shown in this report). The enhanced potency compared to EGF and TGF-α occurs despite the fact that all three EGF family members apparently bind to the same high affinity EGF receptor (4, 9). One possible explanation is differential binding to HSPG. Previous studies have shown that the interaction of two other heparin-binding growth factors, bFGF and vascular endothelial growth factor (VEGF), with cell surface heparan sulfate proteoglycan (HSPG), is an essential
requirement for binding to high affinity receptors and for mitogenic activity (7, 15, 24, 27). These conclusions were based on experiments that used (a) cells mutant in heparan sulfate biosynthesis, (b) cells whose cell surface HSPG was depleted by heparitinase digestion of heparan sulfate, and (c) cells incubated with chlorate to block the sulfation of heparan sulfate. These results suggested that heparin-binding growth factors in general might be dependent on a dual receptor system consisting of HSPG and a high affinity tyrosine kinase receptor that mediates cell signaling. Given that HB-EGF has a strong affinity for immobilized heparin (9), we wanted to determine whether it could bind to cell surface HSPG and whether its bioactivity, in this case the ability to stimulate cell migration, was dependent on interaction with cell surface HSPG. Accordingly, BASMC surface HSPG was altered by heparitinase or chlorate treatment, and a synthetic peptide corresponding to the putative heparin-binding domain of HB-EGF was used to compete for HB-EGF binding to cell surface HSPG. We report here that HB-EGF binds to cell surface HSPG and that the binding of HB-EGF to the EGF receptor on BASMC and its ability to stimulate BASMC migration are considerably diminished when HB-EGF–HSPG interactions are abrogated.

Materials and Methods

Growth Factors

HB-EGF was prepared from U-937 cell conditioned medium as previously described (9, 10). PDGF AB (23) was obtained from Collaborative Research (Bedford, MA). EGF (2 µg) was radiolabeled with Na125I (200 µCi/2 µl; ICN Biomedicals, Costa Mesa, CA) using IODO-BEADS (Pierce, Rockford, IL) as previously reported (10). The specific activity of 125I-HB-EGF was 55,500 cpm/ng, as calculated based on the mitogenic activity of radiolabeled HB-EGF for 3T3 cells compared to HB-EGF standards. 125I-EGF (102,800 cpm/ng) was purchased from Collaborative Research. 125I-labeled HB-EGF (77,000 cpm/ng) was a kind gift of Dr. Brad Otwin (University of Wisconsin).

Cells

BASMC were routinely grown in DMEM supplemented with 10% BCS, 100 µM penicillin and 100 µg/ml streptomycin sulfate (DMEM/10% BCS/PS) as previously described (9, 29). Wild-type (K1) CHO cells and mutant CHO 803 cells, which synthesize ~5–10% of the normal amount of HSPG, were routinely grown in Ham's F12 medium supplemented with 10% BCS as previously described (27).

BASMC Migration

The migration of BASMC was assayed in a Boyden chamber (3, 8). Sub-confluent BASMC were detached from 10-cm culture dishes by incubation with trypsin-EDTA (0.05% trypsin, 0.5 mM EDTA, GIBCO BRL, Gaithersburg, MD) at 37°C. To avoid excessive exposure to trypsin, the incubations were carried out for about 30 min or less. The cells were washed first in DMEM, 10% BCS, and then in binding buffer A (DMEM, 20 mM Hepes, pH 7.4, 0.5% BSA, 1 µM KI in DMEM) and the monolayers were incubated at 4°C for 2 h in binding buffer B containing 125I-HB-EGF (13.3 ng/15-cm dish, 738,000 cpm) or 125I-EGF (9.4 ng/15-mL dish, 966,000 cpm). After the incubation, the cells were washed twice with ice-cold PBS, pH 7.4, containing 0.5% BSA, 1 mM MgCl2, and 1 µM KI, and then twice with ice-cold PBS. Bound 125I-growth factor was cross-linked to cells by the addition of disuccinimidyl suberate (DSS; Pierce, Rockford, IL) at a final concentration of 30 µM in PBS for 20 min at room temperature. After free-reactive DSS was quenched with a large excess of 10 mM Tris-HCl, pH 7.5, 150 mM glycine, the cells were washed with ice-cold PBS, and then scraped and lysed in 400 µl of lysis buffer (1% Nonidet P-40 [Pierce], 1% deoxycholate [Aldrich Chem. Co., Milwaukee, WI], 0.1% SDS, 1% 0.1% aprotinin [Boehringer Mannheim Corp., Indianapolis, IN], 1 mM PMSF [Pierce], 2 mM EDTA, 10 mM sodium fluoride, 10 mM sodium pyrophosphate, 0.4 mM sodium orthovanadate, 10 mM iodoacetamide [Aldrich] in PBS). Samples (30 µl) were subjected to SDS-PAGE and 125I-labeled bands were visualized and quantitated by analysis using a PhosphorImager device (Molecular Dynamics, Sunnyvale, CA).

Characterization of a Synthetic Peptide Corresponding to the Heparin-binding Domain of HB-EGF

A synthetic peptide corresponding to the putative 21–aminio acid heparin-binding sequence KKKKKGRLGKKRRDPCRSKYYK of human HB-EGF (amino acids 93-113 of the 208 residue precursor [21]) was chemosynthetically synthesized using an Applied Biosystems Peptide Synthesizer (courtesy of Margaret Ehrhardt [Brigham and Women's Hospital, Boston, MA]). To determine the effect of P21 on the binding of HB-EGF to heparin-Sepharose, 40 µl of 50% heparin-Sepharose (Pharmacia) in 10 mM Tris-HCl, pH 7.4, 0.2 M NaCl, were mixed with 0.1 µg of 125I-HB-EGF and 50 µl of 10% BCS in 0.2 M NaCl, and the mixture was incubated on a shaker at 4°C for 1 h. The wells were then washed three times with ice-cold binding buffer A and twice with ice-cold PBS/0.5% BSA. Total binding was measured by determining the amount of radioactivity eluted with a wash of 2 M NaCl, 20 mM Na-acetate, pH 4.0 (18, 19, 27).

Binding Assays

The ability of 125I-HB-EGF to bind to BASMC and CHO cells was measured in binding assays carried out in 6-well plates. Briefly, confluent cells (3 × 105 cells/well) in each well were washed with ice-cold PBS/0.5% BSA, and incubated for 10 min at 4°C with 750-µl ice-cold binding buffer A. 125I-EGF (0.125 ng/750 µl/well, 12,800 cpm) or 125I-HB-EGF (0.25 ng/750 µl/well, 13,900 cpm) were added to wells of the plates and the plates were incubated on a shaker at 4°C for 2 h. The wells were then washed three times with ice-cold binding buffer A, and twice with ice-cold PBS/0.5% BSA. Total binding was measured by determining the amount of radioactivity eluted with a wash of 2 M NaCl, 20 mM Na-acetate, pH 4.0 (18, 19, 27).
mixed with 40 µl of the same buffer containing increasing amounts of P21 and constant amounts of 125I-HB-EGF (0.25 ng, 13,900 cpm), 125I-EGF (0.125 ng, 12,800 cpm), and 125I-bFGF (0.27 ng, 20,800 cpm). The mixtures were incubated for 5 min at room temperature, after which the heparin-Sepharose was washed four times with 400 µl of 10 mM Tris-HCl, pH 7.4 and counted in a gamma counter (Beckman Gamma 5500). To monitor the effects of P21 on the stimulation of migration by HB-EGF, BASMC (60-70% confluent) were trypsinized, washed with DMEM, 10% BCS, incubated with binding buffer A, incubated with 25 µg/ml P21 in binding buffer A for 10 min at 37°C, and washed three times with the binding buffer to remove nonbound P21. The BASMC pretreated with P21 were analyzed for the response to migration factors in a Boyden chamber. To monitor the effects of P21 on cross-linking, BASMC were incubated with 25 µg/ml P21 and either 125I-HB-EGF or 125I-EGF on ice, and cross-linking was measured as described above.

Results

HB-EGF Binds to Cell Surface HSPG

HB-EGF binds to immobilized heparin (2, 9, 10). To demonstrate that HB-EGF can also bind to cell surface HSPG, the ability of 125I-HB-EGF to bind to CHO cells was measured in several ways (Fig. 1). CHO cells possess cell surface HSPG but little if any EGF receptor. The binding of 125I-HB-EGF to mutant CHO cells which are deficient in HSPG biosynthesis was diminished by ~70% in comparison to the binding of 125I-HB-EGF to wild-type CHO cells (Fig. 1 A). In addition, treatment of CHO cells with heparitinase which degrades cell surface HSPG, or chlorate which inhibits HSPG sulfation, diminished 125I-HB-EGF binding by ~80% (Fig. 1 B). Taken together, it appears that the binding of HB-EGF to CHO is markedly reduced in the absence of HSPG and that therefore HB-EGF binds to CHO cell surface HSPG. On the other hand, 125I-EGF did not bind at all to CHO cells consistent with its inability to bind to immobilized heparin (2) (Fig. 1 C).

Since we were interested in analyzing the role of HSPG in modulating HB-EGF biological activity towards SMC, 125I-HB-EGF binding to BASMC was measured. Unlike CHO cells, SMC mutant in HSPG biosynthesis are not available and these cells possess EGF receptor (9). Nevertheless, both heparitinase and chlorate diminished total binding of 125I-HB-EGF to BASMC by 80% (Fig. 2), an amount similar to that produced by these treatments of CHO cells. Thus, it is apparent that HB-EGF binds to BASMC HSPG in addition to the EGF receptor. On the other hand, heparitinase did not diminish at all 125I-EGF binding to BASMC while chlorate reduced 125I-EGF binding by 25%. Taken together, these results suggest that EGF, unlike HB-EGF, binds very poorly, if at all, to BASMC HSPG.

The Ability of HB-EGF to Stimulate BASMC Migration Is Dependent on Interactions with Cell Surface HSPG

It had been previously shown that HB-EGF is a potent mitogenic factor for BASMC, with half-maximal activity at 100 pg/ml (9). The mitogenic activity of HB-EGF was comparable to PDGF but its specific activity was 30-40 times greater than EGF. Using a Boyden chamber migration assay, it is apparent that this is true for migration as well. HB-EGF is a potent stimulator of BASMC migration, with a half-maximal stimulation at 150 pg/ml (Fig. 3), and 100 pg/ml HB-EGF is as active as 330 pg/ml PDGF AB and 3.3 ng/ml EGF. Since HB-EGF, but not EGF, binds to HSPG, the relationship between growth factor-mediated biological activities and interactions with cell surface HSPG was subsequently analyzed. Migration rather than mitogenic activity was measured in this analysis because the shorter duration of migration assays (4 h) compared to mitogenic assays (72 h) minimized the amount of time needed to measure the effects of growth factors on BASMC treated in various ways. Ac-
Figure 3. Stimulation of BASMC migration. Migration was measured in a Boyden chamber assay. BASMC were exposed to increasing concentrations of HB-EGF (closed circles), PDGF AB (open circles), and EGF (triangles) in the lower wells of a Boyden chamber and the number of cells per well migrating across the polycarbonate membrane in the chamber was measured. Each point is the average of quadruplicate measurements. The background migration in the absence of growth factor was 20 ± 5 cells/well.

Accordingly, stimulation of migration by HB-EGF was measured on BASMC that had been incubated for either 3 h with heparitinase or for 48 h with chlorate (Fig. 4 A). Both treatments markedly inhibited the ability of HB-EGF to stimulate migration at all concentrations tested. At the concentration of maximal HB-EGF stimulation, 1 ng/ml, heparitinase or chlorate-treated BASMC migration was diminished by 80%. 1 ng/ml HB-EGF was needed to stimulate the migration of treated BASMC to the same extent as did 150 pg/ml HB-EGF for untreated BASMC. Indeed, the ability of HB-EGF to stimulate BASMC depleted of HSPG was more comparable to that typically seen for EGF on BASMC with a normal complement of cell surface HSPG. Taken together, these results suggested that the ability of HB-EGF to induce migration of BASMC is reduced markedly if the cells are diminished in available or intact cell surface HSPG. For comparison and as a control for SMC viability, the activity of PDGF AB, a potent stimulator of SMC migration (23), was measured on BASMC treated with heparitinase or chlorate (Fig. 4 B). The ability of PDGF AB to stimulate BASMC migration was not diminished at all by heparitinase suggesting that this treatment was not toxic for SMC nor was the availability of cell surface HSPG an absolute requirement for migration. Chlorate, on the other hand, did diminish PDGF AB-stimulated migration but by only ∼20%.

Inhibition of HB-EGF-stimulated BASMC Migration by a Heparin-binding Domain Synthetic Peptide Analogue

Another approach to determining the importance of HB-EGF interactions with SMC HSPG would be to interfere with the ability of HB-EGF to bind to cells via its heparin-binding domain. A putative heparin-binding domain in human HB-EGF has been identified by site-directed mutagenesis and peptide fragment studies (Thompson, S., S. Higashiyama, K. Wood, M. Klagsbrun, and J. Abraham, manuscript in preparation). The results of this study are summarized in Fig. 5 A which shows a putative heparin-binding domain of 21 amino acids (amino acids 31-51 of the mature 86-amino acid form of HB-EGF [10]). A 21-amino acid synthetic peptide corresponding to this domain, designated as P21, was prepared and tested for the ability to interfere with the binding of HB-EGF to heparin-Sepharose (Fig. 5 B). The peptide inhibited half-maximally binding of 125I-HB-EGF to heparin-Sepharose at a concentration of ∼2 mg/ml. On the other hand, 125I-EGF did not bind to heparin-Sepharose at all consistent with previous observations (13) and therefore was unaffected by P21. Interestingly, the binding of 125I-bFGF, a growth factor with a very strong affinity for heparin and HSPG (13), to heparin-Sepharose
Figure 5. Inhibition of HB-EGF binding to heparin-Sepharose by a synthetic peptide corresponding to a putative heparin-binding domain of HB-EGF. (A) The sequence of mature HB-EGF (9, 10) with the location of its 21-amino acid putative heparin-binding domain (residues 31-51 of HB-EGF) outlined in gray. (B) The synthetic peptide (P21) corresponding to residues 31-51 was tested in a dose-dependent manner for its effect on the binding of 125I-HB-EGF (closed circles), 125I-EGF (open circles) or 125I-bFGF (triangles) to heparin-Sepharose as described in Materials and Methods. Binding of 125I-growth factor in the presence of P21 is plotted relative to control values in the absence of P21 except 125I-EGF which is plotted relative to 125I-HB-EGF. Under the conditions of these experiments, the control binding of 125I-bFGF (0.27 ng, 20,800 cpm), 125I-HB-EGF (0.25 ng, 13,900 cpm), 125I-EGF (0.125 ng, 12,800 cpm) to heparin-Sepharose in the absence of P21 was 15,670, 5,600, and 50 cpm, respectively.

Figure 6. Effect of the synthetic peptide, P21, on BASMC migration. Stimulation of BASMC migration in response to increasing concentrations of (A) HB-EGF or (B) PDGF AB, was measured in the absence of (closed circles) and in the presence of 25 μg/ml P21 (open circles). Each point is the average of quadruplicate values. The background migration in the absence of HB-EGF and PDGF AB was 23 ± 6 and 12 ± 4 cells/well, respectively.

Effects of Heparitinase, Chlorate, and P21 on Receptor Cross-linking

125I-EGF and 125I-HB-EGF could both be cross-linked to BASMC to form labeled complexes of ~170 kD (Fig. 7). These complexes co-migrated with ligand-EGF receptor complex standards prepared by cross-linking 125I-EGF or 125I-HB-EGF to A431 cells (not shown). 125I-HB-EGF cross-linking was inhibited by excess cold EGF and 125I-EGF cross-linking was inhibited by excess cold HB-EGF supporting the previous conclusion that EGF and HB-EGF bind to the same EGF receptor (9) (not shown). The synthetic peptide, P21, inhibited the cross-linking of 125I-HB-EGF (lane 2) to BASMC by ~75%, comparable in degree to the inhibition of cross-linking caused by chlorate (lane 3) and heparitinase (lane 4) treatment of BASMC. On the other hand, P21 did not inhibit 125I-EGF cross-linking to BASMC (lane 6) nor did heparitinase (lane 8). Chlorate inhibited
Figure 7. Cross-linking of HB-EGF and EGF to the EGF receptor on treated and untreated BASMC. BASMC were either untreated or exposed to heparitinase, chlorate, or 25 μg/ml of the synthetic peptide, P21. The cells were incubated with either 125I-HB-EGF (lanes 1–4) or 125I-EGF (lanes 5–8). After cross-linking, samples were subjected to SDS-PAGE, and bands were visualized (Top) and quantitated by phosphor-imaging (Bottom) as described in Materials and Methods. The growth factor complexes formed comigrated with an EGF-EGF receptor complex standard prepared by cross-linking 125I-EGF to A431 cells (data not shown).

125I-EGF cross-linking slightly, by ~20% (lane 7), consistent with the EGF-BASMC binding studies shown in Fig. 2.

In conclusion, using several different approaches, it is apparent that the binding of HB-EGF to BASMC surface HSPG is required for its optimal binding to the EGF receptor and that these HB-EGF-HSPG interactions facilitate the ability of HB-EGF to stimulate migration of BASMC.

Discussion

HB-EGF, but not the structurally homologous protein EGF, is a heparin-binding growth factor (2, 9). The fact that both HB-EGF and EGF bind to the EGF receptor (9) combined with the possibility that HB-EGF, but not EGF, binds to cell surface HSPG, affords an excellent opportunity to ask the question of whether HB-EGF binding to HSPG modulates its bioactivity. The ability of HB-EGF to bind to cell surface HSPG can be demonstrated by using mutant CHO cells that synthesize only 5–10% of their normal cell surface HSPG. These HSPG deficient cells bind 125I-HB-EGF only 30% as well as do the wild-type CHO cells. In addition, binding to wild-type CHO cells is diminished by 80% after treatment of cells with heparitinase which destroys cell surface HSPG and with chlorate which blocks sulfation of HSPG. Since we have found that the CHO cells express little if any EGF receptor, these experiments suggest strongly that HB-EGF is capable of binding to cell surface HSPG. On the other hand, 125I-EGF does not bind to CHO cells suggesting a lack of interaction with cell surface HSPG.

To analyze the effects of HSPG on HB-EGF bioactivity, we chose to study BASMC because of the previous demonstration that HB-EGF is a far more potent mitogenic factor for these cells than EGF (9) and the subsequent demonstration (Fig. 3) that this enhanced potency is true for HB-EGF stimulation of BASMC migration as well. It is apparent that HB-EGF binds to BASMC HSPG since treatment of these cells with heparitinase or chlorate diminishes 125I-HB-EGF binding by ~80%. Using the migration assay, it appears that methods that interfere with HB-EGF-SMC HSPG interactions also inhibit HB-EGF activity. For example, the ability of HB-EGF to stimulate BASMC migration is considerably diminished, by ~70–80%, when these cells are treated with heparitinase or with chlorate. Furthermore, a synthetic peptide, P21, corresponding to the putative heparin-binding domain of HB-EGF (a 21-aa stretch just upstream and slightly overlapping the EGF-like domain [Thompson, S., S. Higashiyama, K. Wood, M. Klagsbrun, and J. Abraham, manuscript in preparation] that blocked HB-EGF, but not bFGF, binding to heparin-Sepharose), inhibited HB-EGF stimulation of BASMC migration by ~60%. As a control for the possibility that heparitinase, chlorate, or P21 treatment might be toxic to BASMC and inhibit migration directly, it was demonstrated that heparitinase and P21 did not diminish at all, while chlorate diminished slightly, the potent ability of PDGF AB to stimulate BASMC migration. It should be pointed out, however, that chlorate treatment resulted consistently in a 20–25% inhibition of binding and migration, possibly due to cell toxicity caused by the long exposure, 48 h, of BASMC to low cysteine media. The lack of PDGF dependence on HSPG-binding is consistent with the relatively low affinity of PDGF for heparin-Sepharose. PDGF which has a pI of 9.8, is eluted from heparin-Sepharose with 0.5 M NaCl, typical of basic proteins that bind to heparin due to cation-anion charge interactions rather than to any intrinsic heparin affinity (13). Treatments aimed at abrogating growth factor-HSPG interactions also had no effect on the relatively poor ability of EGF to stimulate BASMC migration consistent with the inability of EGF to bind to immobi-
lized heparin or cell surface HSPG. Taken together, it appears that if HB-EGF is prevented access to intact BASMC HSPG by either HSPG degradation, HSPG desulfation, or by a peptide that competes for HB-EGF binding to HSPG, it is no longer optimally bioactive and acts more like EGF, a relatively poor stimulator of BASMC migration.

How do HB-EGF-HSPG interactions modulate bioactivity? In cross-linking experiments in which formation of growth factor-EGF receptor complexes can be visualized and quantitated, it was found that heparitinase-, chlorate-, or P21-treated BASMC display considerably diminished, by ~70–80%, HB-EGF covalent cross-linking to the tyrosine kinase EGF receptor. On the other hand, heparitinase and P21 had no inhibitory effect and chlorate a slight inhibitory effect on EGF cross-linking to the BASMC EGF receptor consistent with the relative inability of EGF to bind to immobilized heparin or to cell surface HSPG and consistent with the lack in EGF of the heparin-domain present in HB-EGF. These results suggest that the ability of HB-EGF, but not EGF, to bind to the EGF receptor is dependent on its ability to bind to cell surface HSPG. Dependence of HB-EGF binding and bioactivity on a dual receptor system consisting of cell surface HSPG, perhaps acting as low affinity receptors, and the EGF tyrosine kinase receptor, is consistent with results found previously for two other heparin-binding growth factors, bFGF and VEGF (7, 15, 24, 27).

The actual mechanisms by which binding to cell surface HSPG modulates the bioactivity of heparin-binding growth factors such as HB-EGF, bFGF, or VEGF are not well understood. Possible mechanisms are that: (a) HSPG concentrates heparin-binding growth factors on the cell surface making them more available to the cell (13), (b) HSPG stabilizes, or alters the conformation of heparin-binding growth factors and/or their receptors, thereby increasing ligand-receptor affinity (27), (c) HSPG stabilizes heparin-binding growth factor dimerization in turn facilitating receptor dimerization as suggested for bFGF (22), and (d) HSPG lowers the off-rate component of binding to high affinity receptor by heparin-binding growth factors. For example, it has been demonstrated that the loss of HSPG increases the off-rate of binding of bFGF to its high affinity tyrosine kinase receptor (20).

The identification of growth factors that are potent stimulators of SMC migration and proliferation could be significant in delineating the causes of pathological SMC hyperplasia that occurs in restenosis after angioplasty, in atherosclerosis, and in hypertension (14, 25). It has been proposed that growth factors such as PDGF and bFGF may be involved in the SMC migration and proliferation that occurs, for example, after intra-arterial injury induced by a balloon catheter (6, 16, 21). HB-EGF might play a role in these migration and proliferative processes as well since preliminary analysis indicates that the levels of HB-EGF mRNA rise markedly as early as 2 h postballoon injury in the rat carotid artery (Chao, S., M. Reidy, S. Dluz, D. Damm, J. Abraham, and M. Klagsbrun, manuscript in preparation). Analysis of how HB-EGF structure affects its biological activity for SMC, for example via heparin-binding, might be useful in learning how SMC growth is regulated and in developing strategies for antagonizing SMC migration and proliferation under pathological conditions.

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