Soluble heparin-binding peptides regulate chemokinesis and cell adhesive forces

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Chon, John H, and Elliot L. Chaikof. Soluble heparin-binding peptides regulate chemokinesis and cell adhesive forces. *Am J Physiol Cell Physiol* 280: C1394–C1402, 2001.—The ability of a soluble heparin-binding peptide sequence derived from fibronectin to modulate the adhesion and chemokinetiic migration behavior of arterial smooth muscle cells was assessed using a novel glass microsphere centrifugation assay and automated time-lapse fluorescence videomicroscopy, respectively. Treatment of cells grown on fibronectin-coated substrates with the soluble heparin-binding peptide resulted in the disassembly of focal adhesions, as assessed by immunohistochemical staining. These observations were consistent with an observed dose-dependent two- to fivefold reduction in cell-substrate adhesive strength (P < 0.001) and a biphasic effect on migration speed (P < 0.05). Moreover, heparin-binding peptides induced a twofold reduction (P < 0.01) in two-dimensional cell dispersion in the presence of a non-heparin-binding growth factor, platelet-derived growth factor-AB (PDGF-AB). Heparin-binding peptides were unable to mediate these effects when cells were grown on substrates lacking a heparin-binding domain. These data support the notion that competitive interactions between cell surface heparan sulfates with heparin-binding peptides may modulate chemokinetic cell migration behavior and other adhesion-related processes.

heparan sulfates; focal contact; extracellular matrix

CURRENT EVIDENCE SUGGESTS that cell surface heparan sulfate proteoglycans (HSPG) promote focal adhesion formation, thereby enhancing cell-substrate interactions. The role of cell surface heparan sulfates in the formation of focal adhesions was initially suggested by the inability of two mutant cell lines that lacked or had altered HSPGs to form focal adhesions in response to a fibronectin substrate (10, 21). Before these observations, Woods et al. (37) had demonstrated that substrata coated with the isolated cell-binding domain of fibronectin were not sufficient for complete cell adhesion; cells attached and spread but did not form focal contacts unless heparin-binding fibronectin sequences were added. Others (4, 35) have also observed that endothelial cells, vascular smooth muscle cells, and human fibroblasts are able to spread on cell-binding RGD containing fibronectin fragments but are unable to form actin stress fibers or focal contacts if the glycosaminoglycan (GAG)-binding domain is absent. Pre-treatment of cells with heparitinase I and III also blocks focal contact formation (35, 39).

To date, most studies that have investigated the potential of soluble peptides to effectively compete for cell surface adhesion receptors have focused on peptides or protein fragments that contain integrin-binding RGD sequences (14, 17). For example, Wu et al. (40) demonstrated that an RGD-containing peptide was capable of enhancing chemokinetic cell motility on substrates containing high surface concentrations of fibronectin. These results were consistent with an observed reduction in the strength of short-term adhesive interactions in the presence of a competing integrin-binding peptide. Because the interaction of cell surface HSPGs with matrix-bound heparin-binding domains promotes focal adhesion formation, we speculated that the presence of soluble heparin-binding peptides might also influence cell locomotion through direct competitive interactions. Specifically, we have investigated the ability of heparin-binding fragments to alter cell adhesion strength and chemokinetic cell motility by either limiting the formation of focal adhesions and/or by inducing their disassembly. We have observed that a heparin-binding peptide derived from fibronectin is capable of inducing a dose-dependent decrease in cell adhesive strength and a biphasic effect on chemokinetic cell migration speed. This phenomenon appears to be related, in part, to the disassembly of focal adhesions.

MATERIALS AND METHODS

Cell culture techniques. Immortalized rat pulmonary arterial smooth muscle cells were a gift of Dr. A. Rothman (University of California San Diego Medical Center, San Diego, CA; see Ref. 29). Cells were grown in growth media composed of medium 199 (M199; Mediatech) supplemented with 10% FBS (HyClone), 2 mM L-glutamine (GIBCO-BRL Life Technologies), and 1% antibiotic-antimycotic mixture (GIBCO-BRL Life Technologies). Cell quiescence was established by incubation for 24 h in M199 with 0.5% FBS (quiescence medium). All migration and adhesion assays were performed in serum-free medium. Serum-free medium con-
sisted of MCDB-104 (GIBCO-BRL Life Technologies) supplemented with 1 mM CaCl₂, 10 mg/l insulin, 6.7 μg/l sodium selenite, 3.5 mg/l transferrin, 2 mM L-glutamine, and 1% antibiotic-antimycotic mixture.

Peptides. All peptides were synthesized in the Emory University Winship Cancer Center Microchemistry Center. The heparin-binding peptides used in this study are derived from the known primary amino acid sequence of fibronectin designated as FN-C/H-V (WQPPRARD) and scrambled FN-C/H-V (RPQIPWPAR; see Refs. 24 and 39). In selective cases, an additional tyrosine or cysteine residue was added to the COOH-terminus of the peptides to facilitate iodination or fluorescein labeling, respectively. The sequence for the scrambled FN-C/H-V peptide is identical to that reported by Huebsch et al. (18, 19). Human plasma fibronectin (pFN; Sigma), the 110-kDa fibronectin fragment (FN-110; Upstate Biotechnology), which has an RGD cell-binding domain but lacks a heparin-binding domain, collagen type I (Vitrogen), and recombinant human platelet-derived growth factor-AB (PDGF-AB; GIBCO-BRL) were obtained from commercial sources.

Protein adsorption assay. Fibronectin, collagen type I, and heparin-binding peptides were radiolabeled with 125I by using Iodobeads (Pierce) following the manufacturer’s protocol. Both the percentage of iodine incorporation and specific activity were determined. We have previously reported detailed methods for determining the fibronectin adsorption profile on non-tissue culture-treated polystyrene discs (7, 8). The surface density (d) of other proteins or peptide sequences adsorbed on glass coverslips and glass microspheres (d 30–50 μm; Polysciences) was ascertained in a similar fashion. Briefly, glass or polystyrene surfaces were incubated with protein or peptide for 24 h at 4°C followed by three washes with PBS, blocking with 1% BSA for 45 min at room temperature, and three more washes with PBS. Adhesion and migration assays, as well as correlative immunohistochemical staining studies, were conducted on a variety of fibronectin-coated substrate types (e.g., glass vs. nontissue culture poly- styrene) and configurations (e.g., spherical vs. planar surfaces). The determination of an absolute fibronectin surface density on each surface type ensured that consistent correlations between assays could be ascertained.

Immunostaining. Focal adhesion formation and disassembly in the presence of soluble heparin-binding peptides were examined in cells cultured on fibronectin-coated glass coverslips. Coverslips (Fisher Scientific) were incubated with pFN for 24 h at 4°C, followed by three washes with PBS, blocking with 1% BSA for 45 min at room temperature, and three more washes with PBS. Cells at 80% confluency were detached and placed inverted in a 12-well plate prefiltered with 0.1% BSA in PBS. Typically, beads covered >95% of the well surface area, which represents ~33,000 beads/well. The plate was centrifuged for 1 min at speeds ranging from 500 to 3,500 rpm, and each well then was viewed under a microscope using a ×4 objective. The percentage of beads remaining attached to the cell layer was characterized by computing the fraction of the well surface that remained covered by beads. This was facilitated by visualizing the wells with high ambient light and performing routine image analysis on the captured image (IPLab Spectrum; Signal Analytics).

The centrifugal force at which 50% of the beads are detached from the cell surface was computed by plotting the percentage of attached beads as a function of centrifugal force. The Levenberg-Marquardt method for nonlinear least squares fitting was used to fit the data to

\[ f = \frac{f_0}{1 + \exp[b(F - F_{50})]} \]

where the experimental variables include the fraction of beads remaining on the cell monolayer (f) and the centrifugal force (F, in g). The parameters that are fitted to the logistic equation are the fraction of well surface covered by beads at zero force (f₀), the decay slope (b), and the force (g) at which 50% of the beads are detached (F₅₀). F₅₀ can also be expressed in terms of millinewtons per square centimeter if an average contact area between beads and cell monolayer is assumed. Use of this equation assumes that the bead population has a normal distribution of adhesion properties that arise from heterogeneities in bead size, ligand number, and binding characteristics. With the application of a detachment force, the adherent bead fraction is expected to decrease in a sigmoidal fashion (11, 15, 30). The correlation coefficient (R²) exceeded 0.90 for all curve fits.

Single-cell migration assay. All migration assays were performed using a computer-assisted, fluorescence videomicroscopy system, as described previously (7, 8). Briefly, cells at ~80% confluency were labeled with 0.625 μg/ml 1,1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) in quiescence medium for 24 h. Cells were then seeded in serum-free medium at a density of ~1,000 cells/cm² in non-tissue culture-treated polystyrene multiwell plates coated at a defined surface density with a specific matrix protein. Cells were observed for 24 h with images obtained at 30-min intervals. The images were analyzed to determine the location of the centroids of each cell at each observation time point.
Average squared displacement as a function of time interval \(n\Delta t\) for each cell was calculated on an Excel spreadsheet (Microsoft) using the spatial coordinates obtained from image processing and sorted using a cell tracking program described in detail elsewhere (8). Displacements were converted from pixels to micrometers with the factor \(C = 1.00\ \mu\text{m/pixel}\), measured using a stage micrometer. IPLab Spectrum uses square pixels, negating the need to include a correction factor for either horizontal or vertical directions. Squared displacements from \([x(t), y(t)]\) to \([x(t + i\Delta t), y(t + i\Delta t)]\) were calculated using the following equation

\[
d^2_{t\rightarrow t+i\Delta t} = C^2[(x(t + i\Delta t) - x(t))^2 + (y(t + i\Delta t) - y(t))^2]
\]

To take advantage of all positional information available, overlapping intervals were used, and mean square distance \((d^2)\) was calculated for all possible time intervals (0.5–24 h in 30-min increments). The resulting list of \(d^2\) as a function of migration time, \(t\), was then fitted to a persistent random walk model described by the equation

\[
\langle d^2 \rangle = nS^2P[t - P(1 - e^{-Pt})]
\]

where \(S\) is the cell speed in micrometers per hour, \(P\) is the persistence time in hours, and \(n\) is the number of dimensions in which the cell migrates (26). The experimental data were fit to the model using the Levenberg-Marquardt method. The dispersion coefficient (\(\mu\)) was determined as

\[
\mu = \frac{1}{n} S^2 P
\]

with \(n = 2\) for motility on a two-dimensional surface. By applying this method to each of the cells tracked, we were able to determine the mean cell speed and persistence time as well as the dispersion coefficient with standard errors for each assay.

Any cell that migrated with a persistence time of <30 min and that traveled <20 \(\mu\text{m}\) from the original starting point over the 24-h period was considered immotile. The application of these criteria to distinguish immotile from motile cells was not sensitive to the exact cutoff value for \(S\) or \(P\). Typically, \(P << 0.01\ h\) and \(d^2 < 100\ \mu\text{m}^2\) were observed for immotile cells. In the presentation of our results and in the subsequent discussion, we define cell speed (\(S\)) as the mean speed of all motile cells. Typically, 100–250 cells were observed for each condition under investigation. A more complete description of the methodology of fluorescence cell tracking, algorithm development for automated data analysis, the potential effects of DI on cell viability and behavior, and validation of this technique compared with phase-contrast-based methods can be found elsewhere (7,8).

**RESULTS**

Adsorption of heparin-binding peptides to fibronectin-coated substrates. To ensure that soluble heparin-binding peptide sequences were not adsorbed to any significant degree on test well surfaces, \(^{125}\text{I}-\text{labeled peptides were used in an adsorption study. Glass coverslips, coated with human pFN and blocked with BSA, were incubated overnight with 100 \(\mu\text{M}\) peptide solutions. Minimal adsorption of FN-C/H-V was observed on either fibronectin- or BSA-adsorbed glass coverslips (<0.5 nmol/cm\(^2\) or <0.4% of total added peptide).**

*Fig. 1. Disassembly of focal adhesions by competitive inhibition with heparin-binding peptide FN-C/H-V (WQPPRARI). Pulmonary artery smooth muscle cells (PAC1) seeded on plasma fibronectin (pFN)-coated glass coverslips were stained for vinculin to visualize focal adhesions. The concentrations used included no treatment (A), 1 \(\mu\text{M}\) FN-C/H-V (B), 100 \(\mu\text{M}\) FN-C/H-V (C), and 100 \(\mu\text{M}\) scrambled FN-C/H-V (RPQIPWAR; D).*
at the end of actin bundles and around the edge of the cell membrane, remained visible. However, in the presence of 100 μM FN-C/H-V peptide, vinculin clusters were no longer detectable. Rather, uniform staining of the cell, but not the nucleus, by the anti-vinculin antibody was observed. The treatment of cells with the scrambled FN-C/H-V peptide did not appear to have any detectable effect on the formation of focal adhesions.

Smooth muscle cell adhesion is inhibited in a dose-dependent manner by heparin-binding peptides. The adhesive strength between cells and adsorbed fibronectin-coated glass microspheres, in the presence or absence of test peptide and the respective scrambled sequence, was defined using a modified centrifugal bead assay. This assay facilitated the generation of an adhesion profile as a function of soluble peptide concentration, and logistic regression allowed expression of data in terms of F50 (Fig. 2 and Table 1).

In the presence of FN-C/H-V peptide, a significant dose-dependent decrease in cell adhesion strength to fibronectin-coated microspheres was observed (P < 0.001). Indeed, when cells were treated with 1 μM of FN-C/H-V peptide, adhesive strength decreased by 50%. In the presence of 100 μM FN-C/H-V, a fivefold reduction in adhesive strength was observed. The addition of the scrambled peptides had no appreciable effect on these adhesive interactions. Cell detachment or cell death was not observed at any of the tested peptide concentrations (data not shown).

Soluble heparin-binding peptides modulate chemokinetic smooth muscle cell migration. To ensure comparable fibronectin surface densities, the adsorption isotherms of fibronectin on glass and non-tissue culture-treated polystyrene surfaces were determined and noted to be similar on both surfaces (Fig. 3). Conformational differences between fibronectin adsorbed on glass and polystyrene may exist; however, the effect on heparan sulfate binding is likely small. Furthermore, all assays were performed using surfaces coated at a concentration of pFN that exceeded monolayer coverage (0.5 μg/cm²).

To assess the effect of competitively inhibiting the interaction between cell surface heparan sulfate and the underlying fibronectin substrate, soluble heparin-binding peptides were added to the medium during the migration assay. Chemokinetic cell migration behavior was characterized at concentrations of soluble peptide ranging from 1 to 100 μM. In the presence of FN-C/H-V peptide, a biphasic dependence of cell speed with soluble peptide concentration was observed (P < 0.01; Fig. 4). At a FN-C/H-V concentration of 1 μM, the speed of the migrating cells increased by 20% (P < 0.05), whereas at 100 μM it decreased by nearly 15%.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>F50, g</th>
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<tbody>
<tr>
<td>None</td>
<td>490 ± 100</td>
</tr>
<tr>
<td>FN-C/H-V (1 μM)</td>
<td>262 ± 35*</td>
</tr>
<tr>
<td>FN-C/H-V (100 μM)</td>
<td>89 ± 13*</td>
</tr>
<tr>
<td>Scrambled FN-C/H-V (100 μM)</td>
<td>572 ± 51</td>
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Values are means ± SE. FN, fibronectin; F50, force at which 50% of the beads are detached. *P < 0.005.

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Table 1. Cell-substrate adhesive strength in the presence or absence of FN-C/H-V or scrambled peptide sequences

![Fig. 2. Effect of heparin-binding peptides on cell adhesion. The adhesive strength of PAC1 cells was assessed before and after treatment with heparin-binding peptides. A: cell-substrate adhesion profile was determined in the absence of added peptide. B: cells were exposed to 100 μM/ml of FN-C/H-V (●) or scrambled peptide (○). C: adhesive strength of PAC1 cells assessed as a function of FN-C/H-V concentration. Cells were incubated with 1 (●) or 100 (△) μM/ml of FN-C/H-V. The correlation coefficient (R²) exceeded 0.90 for all curve fits.](http://ajpcell.physiology.org/)
Both of these values were compared with baseline measurements. Of interest, the percentage of cells that were motile decreased with increasing FN-C/H-V concentration; however, statistical significance was not achieved. For example, in the presence of 100 μM FN-C/H-V, the percentage of motile cells decreased by nearly 25%. No significant effect was observed with the addition of scrambled FN-C/H-V sequence over a similar peptide concentration range (data not shown).

Soluble heparin-binding peptides alter cell adhesion and migration by competitive interactions with heparin-binding domains located within insoluble extracellular matrix proteins. We postulated that heparin-binding peptides alter smooth muscle cell adhesion and migration behavior by competitively inhibiting interactions of cell surface heparan sulfates with heparin-binding domains located within insoluble matrix proteins. This hypothesis was tested by defining the effect of the FN-C/H-V sequence on cell adhesion and motility on substrates coated with FN-110, which lacks the heparin-binding domain. Characteristically, focal adhesions are not observed when cells are plated on FN-110. In fact, measured adhesion strength between protein-coated beads and a cell monolayer was significantly lower when beads were coated with FN-110

![Fig. 3. Human pFN adsorption isotherms on glass microspheres (▲) and non-tissue culture-treated polystyrene (○). Surface protein density was determined by measuring adsorbed radioactivity after a 24-h incubation period with the protein solution followed by a washing step.](image)

![Fig. 4. Effect of FN-C/H-V peptide (WQPPRARI) on PAC1 cell migration. PAC1 cells were treated with FN-C/H-V peptide in doses ranging from 0.1 to 100 μM. Cell motility was characterized in terms of cell speed (S; A), persistence time (P; B), dispersion coefficient (μ; C), and percentage of motile cells (%motility; D). Data are presented as means ± SE of at least 100 cells. *P < 0.05. Treatment with the scrambled FN-C/H-V peptide sequence (RPQIPWAR) had no effect on cell motility parameters. Motility parameters in the absence of soluble peptide were: S = 16.36 ± 0.96 μm/h; P = 1.65 ± 0.16 h; μ = 204 ± 18 μm/h; and %motility 63.2 ± 5.1%.](image)
compared with those beads coated with either native fibronectin or collagen type I (Fig. 5). Incubation of cells with the FN-C/H-V sequence had no additional effect on adhesion between beads coated with FN-110 and the underlying cell monolayer. Likewise, no change in cell motility on FN-110-coated surfaces was observed in the presence or absence of the FN-C/H-V sequence (Fig. 6).

**Effect of soluble heparin-binding peptides is enhanced in the context of PDGF-AB-stimulated cell migration.** PDGF-AB does not bind to cell surface heparan sulfates, nor does it alter syndecan, glypican, or cell surface heparan sulfate expression. However, PDGF-AB does increase chemokinetic cell motility through a mechanism probably related to dynamic cytoskeletal assembly processes. We speculated that at increased cell speeds the impact of competing soluble heparin-binding sequences might be enhanced. The effect of FN-C/H-V sequences on PDGF-AB-stimulated cell locomotion is summarized in Fig. 7. Cell speed, persistence time, the dispersion coefficient, and the percentage of motile cells were all increased by PDGF-AB. Notably, treatment with FN-C/H-V had no effect on the proportion of migrating cells. However, a significant reduction in both cell speed and persistence time led to a twofold reduction in the dispersion coefficient ($P < 0.01$), which is indicative of a marked reduction in cell flux across a region of space.

**DISCUSSION**

Recent evidence suggests that cell surface HSPG, in particular, syndecans 1 and 4, are important determinants of cell-matrix adhesive processes. For example, Liebersbach and Sanderson (22) have demonstrated that the expression of syndecan 1 in a human myeloma cell line limits its ability to form tumors in vivo. In addition, Carey et al. (5) have shown that overexpressing syndecan 1 induces cell spreading and the development of microfilament bundles that terminate in focal adhesions. This behavior has been correlated further with an inability of syndecan 1-expressing cells to invade collagen matrixes (34). These reports have suggested that the migration of at least some cell types may require the loss of syndecan 1 HSPG receptors (22, 28).

Although syndecan 1 may have a role in modulating cell-matrix adhesive processes, syndecan 4 is the only HSPG incorporated in focal adhesions, where it colocalizes with $\beta_1$- and $\beta_3$-integrin subunits and secreted fibronectin fibrils (1, 30). Indeed, during the formation of focal adhesions, syndecan 4 becomes clustered into higher-order, self-associating structures that appear to be linked to the cytoskeleton. With this in mind, a current view holds that focal adhesions form in two stages (4). The first stage is dependent on integrin

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**Fig. 5.** Effect of the surface-adsorbed heparin-binding domain on cell adhesion strength. The adhesive strength of PAC1 cells to increasing surface densities of pFN (hatched bars), collagen type I (open bars), and adsorbed 110-kDa fibronectin fragment (FN-110; filled bars) is expressed in terms of the force at which 50% of the beads are detached ($F_{50}$). Data are presented as means ± SD of 4 separate experiments. Cell adhesive strength is significantly reduced in the absence of a matrix-associated heparin-binding domain.

**Fig. 6.** Cell migration on FN-110-coated substrates was investigated in the presence or absence of the FN-C/H-V peptide (WQPPRARI). FN-110 protein surface density had little effect on cell motility parameters (A). Likewise, the addition of FN-C/H-V (100 $\mu$M; open bars, FN-C/H-V vs. filled bars, FN-C/H-V$^+$) did not influence cell locomotion, regardless of FN-110 substrate density (B). Data are presented as means ± SE of at least 100 cells.
activation and clustering, includes aggregation of vinculin, talin, paxillin, tensin, filamin, and α-actinin, and requires the activation of protein kinase C (PKC) and focal adhesion kinase. The second stage is mediated by the interaction of syndecan 4 with heparin-binding domains in the extracellular matrix (ECM) and, likewise, involves PKC signaling (1). In support of a possible role of syndecan 4 in cell locomotion, Woods et al. (38) have recently reported that overexpression of syndecan 4 in CHO-K1 cells limits cell migration. These studies have provided significant insight into a potentially important function of cell surface HSPG. Nonetheless, it bears emphasis that a primary change in substratum adhesiveness could initiate significant changes in motile cell behavior, even in the absence of a requisite change in the surface concentration or binding affinity of heparan sulfate. Specifically, the functional consequences of syndecan 1 or 4 expression must be interpreted within the context of the type, distribution, and spatial density of their respective ligands. Moreover, this process will also be influenced by the ability of these cell surface receptors to effectively interact with available matrix-bound ligands.

It is likely that syndecan 1 and 4 influence cell motility and remodeling processes by direct contact-mediated interactions between core protein-associated heparan sulfate chains with heparin-binding domains found within matrix glycoproteins. For example, at least two domains within each fibronectin monomer bind to heparan sulfate (31). The HEP I site is located at the NH2-terminus, is composed of type I repeat units, and is associated with a relatively weak heparin dissociation constant in the range 10^{-6} to 10^{-5} M (2, 3). The other major heparin-binding site, known as HEP II, is located in three type III repeat units (nos. 12–14) between the cell-binding (RGD) site and the variably spliced IIICS region (domain V) near the COOH-terminus. At least four independent sites consisting of basically charged amino acids may mediate heparin binding within the HEP II domain; none of which overlap with integrin-binding sites. The heparin dissociation constant for HEP II is considerably greater than HEP I, in the range of 10^{-8} to 10^{-7} M (2, 3). Recently, Walker and Gallagher (33) have identified a likely HEP II binding site in heparan sulfate characterized by sequences of N-sulfated disaccharides in which a proportion of the iduronate residues are sulfated at C-2. All of this suggests that competing interactions may take place between heparin-binding proteins with common high-affinity sites on the heparan sulfate chain. Likely, both protein affinity and concentration will determine the end result of these interactions. It has been postulated that competing mechanisms of this kind may play a significant role in the control and integration of cellular responses to a variety of heparin-binding growth factors and matrix proteins. However, confirmatory evidence in support of this hypothesis has been limited.

Our results indicate that heparin-binding peptides derived from the HEP II domain may limit the formation and/or induce the disassembly of focal adhesions, as assessed by immunohistochemical staining. These observations were consistent with an observed dose-dependent decrease in cell-substrate adhesive strength and a biphasic effect on migration speed. The competitive nature of these interactions is also demonstrated by the inability of heparin-binding peptides to mediate these effects when cells were grown on substrates lacking a heparin-binding domain. Finally, a scrambled peptide sequence that contains a high density of cat-
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ionic residues had no significant effect. It is notable that the capacity of heparin-binding peptides to inhibit two-dimensional cell dispersion was enhanced significantly in the presence of a non-heparin-binding growth factor, PDGF-AB. Significantly, PDGF-AB does not affect the expression or shedding of cell surface HSPGs or their interactions with ECM heparin-binding domains (9, 32). PDGF-AB likely influences adhesion strength through a variety of mechanisms, including an effect on integrin expression, activation, or clustering, and may have an impact on other factors that are important to the process of cell migration, such as intracellular force generation or lamellipod extension. Indeed, PDGF-AB significantly increased cell dispersion. Nonetheless, blocking heparan sulfate-matrix interactions through exposure to FN-C/H-V inhibited cell migration, despite the presence of this soluble motogenic factor.

Cell locomotion is a dynamic process involving the formation and breakage of attachments to an underlying substrate (20, 25). In this regard, a large body of data has confirmed that the ability of cells to migrate on a given substrate depends on several variables related to integrin-ligand interactions, including ligand levels, integrin levels, and integrin-ligand binding affinities. Conceptually, integrin-ligand interactions appear to affect the way intracellular pathways integrate, so as to effect and regulate cell migration. As an outgrowth of this notion, Palecek et al. (27) and others (12, 13) have suggested that short-term cell-substratum adhesiveness is rate limiting in determining cell migration speed, as the linkage between integrin and the ECM is altered. This postulate is based on experimental studies that have measured the attachment strength of cells after a 20- to 30-min incubation period with substrates coated with varying surface densities of matrix proteins. In correlating the strength of short-term adhesive interactions with cell migration, intermediate cell attachment strength was associated with both maximal cell speed and directional persistence. Importantly, mathematical models that have evolved in the context of these observations predict that cell speed is a function of both cell motile force and short-term binding strength to the substrate (12, 27). These studies have established an important conceptual framework for understanding the role of adhesive interactions in cell locomotion. Nonetheless, there remain a number of important considerations that have received less attention, including the relative contribution of different time scale adhesive events and the proportionate effect of nonintegrin adhesion receptors in determining cell binding strength to the underlying matrix.

The implications of the experimental studies reported herein are twofold. First, it is clear that heparin-binding domains are critical modulators of both cell adhesion and locomotion. Increasing the surface concentration of the FN-110, which contains a cell-binding (RGD) sequence but lacks a heparin-binding domain, had little effect on cell migration parameters despite the increased availability of surface-bound integrin ligands. Furthermore, loss of the heparin-binding domain was associated with a threefold reduction in cell speed (15 vs. 5 μm/h). Thus, if one accepts that short-term cell-substratum adhesiveness is rate limiting in determining cell migration speed, these results suggest that cell surface heparan sulfate-matrix interactions may be important determinants of the rate-limiting process. It is of interest that Lotz et al. (23) observed a significant increase in adhesive “strengthening” in the first 15 min after initial cell binding to a surface, presumably due to the movement of additional adhesive receptors into the adhesive site and the coupling of the actin cytoskeleton to the adhesion complex. Therefore, our data suggest that dissection of the role of specific cell surface HSPG in these critical early events is worthy of further investigation, particularly with quantitative adhesion assays of high sensitivity. Second, the results of our investigations serve to reemphasize that longer time-scale adhesive events, such as the late clustering of receptors to focal contacts or the formation of ECM or matrix contacts, are relevant to cell movement. Specifically, cell-bead adhesive strength, measured after a 6-h incubation period, was fivefold less to surfaces coated with FN-110 compared with those coated with native fibronectin. Likewise, a fivefold reduction in cell adhesion to fibronectin-coated beads was also observed after the addition of FN-C/H-V with an associated loss in focal adhesions. It is noteworthy that the capacity of fibronectin-derived heparin-binding peptides, and in particular FN-C/H-V, to alter the adhesion of endothelial cells, keratinocytes, and corneal epithelial cells to fibronectin has been investigated extensively, as summarized in prior reports, with little or no effect observed (19, 24, 34). In these reports, standard adhesion assay methodology was used in which cells were incubated for 30–90 min with fibronectin-coated surfaces. Thus short-term cell-substrate-binding assays may not necessarily provide an accurate assessment of all adhesive events that influence cell locomotion, either as a result of inadequate assay sensitivity or as a consequence of being unable to capture all rate-limiting steps that characterize the adhesive process.

The integration of chemical and mechanical signals from the matrix directly influences cell adhesion and motility behavior and, as an end result, tissue morphogenesis. Other mechanisms that effect focal adhesion formation and disassembly have been well documented, including thrombospondin-calreticulin and tenascin C-annexin II interactions, among others (16). Our data suggest that competitive binding interactions between heparin-binding peptides and cell surface GAG chains may also influence wound healing and other processes, in part, by modulating cell adhesion and migration behavior. Although the physiological significance of our observations has not been defined fully, new opportunities may exist for drug development that specifically targets heparan sulfate-matrix interactions.

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