

Role of Cell Surface Heparan Sulfate Proteoglycans in Endothelial Cell Migration and Mechanotransduction

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Endothelial cell (EC) migration is critical in wound healing and angiogenesis. Fluid shear stress due to blood flow plays an important role in EC migration. However, the role of EC surface heparan sulfate proteoglycans (HSPGs) in EC adhesion, migration, and mechanotransduction is not well understood. Here, we investigated the effects of HSPG disruption on the adhesion, migration, and mechanotransduction of ECs cultured on fibronectin. We showed that disruption of HSPGs with heparinase decreased EC adhesion rate by 40% and adhesion strength by 33%. At the molecular level, HSPG disruption decreased stress fibers and the size of focal adhesions (FAs), increased filopodia formation, and enhanced EC migration. Under flow condition, heparinase treatment increased EC migration speed, but inhibited shear stress-induced directionality of EC migration and the recruitment of phosphorylated focal adhesion kinase in the flow direction, suggesting that HSPGs are important for sensing the direction of shear stress. In addition, decreasing cell adhesion by lowering fibronectin density enhanced EC migration under static and flow condition, but did not affect the directional migration of ECs under flow. Based on our results, we propose that HSPGs play dual roles as mechanotransducer on the EC surface: (1) HSPGs–matrix interaction on the abluminal surface regulates EC migration speed through an adhesion-dependent manner, and (2) HSPGs without binding to matrix (e.g., on the luminal surface) are involved in sensing the direction of flow through an adhesion-independent manner. *J. Cell. Physiol.* 203: 166–176, 2005. © 2004 Wiley-Liss, Inc.

The endothelial monolayer serves a number of important homeostatic functions, e.g., the regulation of permeability and vasoactivity, and the mediation of cell adhesion and inflammatory responses. Under pathological conditions such as atherosclerosis and denudation injury following angioplasty and bypass grafting, the loss of endothelial integrity leads to endothelial cell (EC) dysfunction. The recovery of endothelial integrity and vascular wall homeostasis requires endothelial healing, which involves EC migration at the wound edges. The migration process includes the extension of the leading edge, adhesion to the matrix, contraction of the cytoplasm, and release of adhesions at the rear (Lauffenburger and Horwitz, 1996; Sheetz et al., 1998). This process is regulated by cell–matrix interaction and many environmental factors.

EC migration can be modulated not only by chemical stimuli, but also by hemodynamic forces such as fluid shear stress, the tangential component of the hemodynamic forces acting on the vessel wall. Flow channels have been used to investigate the responses of cultured ECs to shear stress *in vitro* because the chemical and mechanical factors can be well controlled. Such *in vitro* studies have shown that shear stress enhances EC migration in wound healing (Ando et al., 1987; Masuda and Fujiwara, 1993; van et al., 1994; Sprague et al., 1997; Albuquerque et al., 2000; Hsu et al., 2001). ECs extend lamellipodia and migrate faster in the flow direction—a mechanotaxis process induced by shear stress (Masuda and Fujiwara, 1993; van et al., 1994; Sprague et al., 1997; Hsu et al., 2001; Li et al., 2002). Mechanotaxis not only promotes EC wound healing in large vessels such as arteries, but may also induce directional angiogenesis in microcirculation, e.g., along the interstitial flow paths or tunnels during wound healing and lymphangiogenesis (Branemark, 1965; Moldovan et al., 2000; Boardman and Swartz, 2003).

By monitoring green fluorescence protein (GFP)-tagged actin and focal adhesion kinase (FAK) in ECs,

we have shown that shear stress induces lamellipodial protrusion and actin polymerization in the flow direction, followed by recruitment of FAK to focal adhesions (FAs) at the leading edge (Li et al., 2002). Recent studies have also shown that microtubule instability, actin dynamics, PI-3 kinase, ERK, and small GTPases Rac and Rho are involved in shear stress-induced EC migration (Albuquerque and Flozak, 2001; Hu et al., 2002; Urbich et al., 2002; Wojciak-Stothard and Ridley, 2003). However, how shear stress is sensed and transmitted from cell surface to intracellular space is unclear.

EC surface is covered by a layer of glycocalyx, a network of glycoproteins and proteoglycans about 0.05–0.5 μm thick (Luft, 1966; Vink and Duling, 1996; Squire et al., 2001), which may function as mechanotransducer for shear stress applied on EC surface. EC surface heparan sulfate proteoglycans (HSPGs), composed of core protein and heparan sulfate-type glycosaminoglycan side chains, may play important roles in the regulation of growth factor binding, blood cell adhesion, and focal adhesion (FA) formation in ECs (Marcum and Rosenberg, 1987; Saksela et al., 1988; Rapraeger et al., 1991; Yayon et al., 1991; Mertens et al., 1992; Tanaka et al., 1998; Bernfield et al., 1999; Couchman and Woods, 1999; Iivanainen et al., 2003). In fibroblasts, HSPGs such as syndecan-4 bind to the heparin-binding domain

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of fibronectin (FN) (Woods et al., 2000), and collaborate with integrins to promote cell adhesion assembly (Woods and Couchman, 1994, 1998; Couchman and Woods, 1999; Longley et al., 1999; Saoncella et al., 1999). However, the role of HSPGs in EC adhesion and migration, especially under physiological flow condition, is not well characterized.

Here, we investigated the roles of HSPGs in EC adhesion and migration under static and flow conditions. To focus on the role of HSPGs in cell–matrix interaction, subconfluent ECs were used in this study. We showed that disruption of HSPGs with heparinase decreased EC adhesion rate and strength, decreased stress fibers and the size of FAs, and enhanced EC migration. Under flow, heparinase treatment increased EC migration speed, but inhibited shear stress-induced directional migration, new FA formation, and the recruitment of phosphorylated FAK in the flow direction. These results suggest that HSPGs play significant roles in mechanotransduction during shear stress-induced EC migration.

MATERIALS AND METHODS

Cell culture

Cell culture reagents were obtained from Gibco BRL (Grand Island, NY) unless otherwise specified. Bovine aortic endothelial cells (BAECs) were isolated from bovine aorta with brief treatment of collagenase. After expansion, the isolated ECs were stained positive for vascular endothelial cadherin (using an antibody from Santa Cruz Biotechnologies, Inc., Santa Cruz, CA), and the cells took in DiI-labeled acetylated low-density lipoproteins (from Molecular Probes, Inc., Eugene, OR), suggesting EC phenotype. BAECs were cultured and expanded in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, and penicillin (100 U/ml)–streptomycin (100 µg/ml) (complete medium). Cell cultures were maintained in a humidified 5% CO₂–95% air incubator at 37°C. All experiments were conducted with BAEC prior to passage 10.

For all experiments, to minimize HSPG synthesis by ECs after heparinase-treatment and avoid introducing HSPG into serum, ECs were trypsinized, resuspended and cultured in low-serum medium, i.e., DMEM supplemented with 0.5% FBS, 2 mM L-glutamine, and penicillin (100 U/ml)–streptomycin (100 µg/ml).

Surface coating and heparinase treatment

All experiments were conducted on FN-coated surfaces. Bovine FN (Fisher Scientific) at 1 µg/cm² (unless otherwise specified) was adsorbed onto culture surfaces (culture wells or glass slides) for 2 h at room temperature. Non-specific binding sites were blocked with 1% bovine serum albumin (Sigma-Aldrich, St. Louis, MO) for 30 min at room temperature. The surfaces were washed with phosphate buffer saline (PBS) after coating.

Heparinase II (Hep II), which cleaves heparin and heparan sulfate in HSPGs (Linhardt et al., 1990), was used to disrupt HSPGs on EC surface. Our pilot studies showed that Heparinase I and Heparinase III had the same effects on BAEC adhesion and migration as Hep II (data not shown). Before experiments, confluent ECs were detached with trypsin, resuspended in low-serum medium with or without Hep II (Sigma-Aldrich) at 0.857 U/10⁶ cells, and incubated at 37°C for 30 min. Then the cells were seeded onto FN-coated surfaces with a density of 2.5 × 10⁴ cells/cm² (for adhesion assays and immunostaining experiments) or 1 × 10⁴ cells/cm² (for migration assays) in the presence or absence of Hep II. In some experiments, ECs were first seeded on FN-coated surfaces, and Hep II was added to the adherent cells to disrupt HSPGs. Applied dosage of Hep II was determined to be sufficient as the higher dosage of 12.0 U/10⁶ cells had same effects on migration speed and adhesion force as the applied dosage (data not shown). This dosage was within the range of

those dosages used previously in other studies (Kapila et al., 1999; Moyano et al., 1999). All our experiments were finished within 6 h after heparinase treatment, which was before the significant synthesis of HSPGs by cells (Yoneda et al., 1995).

Shear stress experiments

A flow system was used to impose shear stress on cultured ECs as described previously with minor modifications (Li et al., 2002). In brief, the glass slide (75 × 25 mm) with EC culture was mounted in a rectangular flow channel (0.025 cm in height, 1.0 cm in width, and 5.0 cm in length) created by sandwiching a silicone gasket between the glass slide and an acrylic plate. The channel has an inlet and an outlet for perfusing the cultured cells. Laminar shear stress was generated by using a MasterFlex peristaltic pump (Cole-Parmer Instrument Company, Vernon Hills, IL) and a damping reservoir. Shear stress was determined by the following equation: $\tau = 6 \mu Q / Wh^2$, where τ is shear stress, μ is viscosity of the medium (0.0084 poise), Q is the flow rate across the flow chamber, and W and h are the width and height of the chamber, respectively. During the flow experiments, the system was kept at 37°C in a constant temperature hood, and circulating medium was ventilated with 95% humidified air with 5% CO₂. All shear stress experiments were conducted at 12 dyn/cm². This level of shear stress is in the physiological range found in human major arteries and has been shown to regulate EC migration (Hsu et al., 2001; Hu et al., 2002; Li et al., 2002). All shear stress experiments include static controls, i.e., ECs cultured on slides not exposed to shear stress. For static controls, BAECs were cultured on Lab-Tek chamber slides coated with FN. The chamber slides were immobilized in a 10 × 10 cm square dish with 5% CO₂ ventilation for time-lapse microscopy.

Time-lapse phase-contrast microscopy

Time-lapse microscopy of cell movement under static and flow conditions was performed using a Nikon inverted microscope (TE300) equipped with 10× objective. A temperature hood was built around the microscope to keep the temperature at 37°C during experiments. Phase-contrast images were collected using a Hamamatsu Orca100 cooled digital CCD camera at 20-min intervals and transferred directly from a frame grabber to computer storage using C-Imaging System software (Compix, Inc., Cranberry Township, PA). Cell outlines were visualized by phase contrast microscopy with 10× objective. A scanning stage allowed us to automatically collect images from different areas of the sample. Dynamic motion of individual cells was analyzed by using Dynamic Image Analysis System (DIAS) software (Solltech, Inc., Oakdale, IA). Cells contacting each other during migration were excluded. The DIAS program was used to determine the centroid position of each cell from the cell outline at each time point, and the cell migration path was generated. The cell migration speed and migration direction were quantified based on cell migration paths. The migration directionality was measured by the average of $\cos\theta$, where θ is the angle between the migration direction and flow direction (or a specified direction under static conditions).

Cell adhesion assay and cell adhesion strength measurement

Cell adhesion assays were performed to determine the contribution of HSPG on cellular adhesion. ECs with or without Hep II treatment in suspension were seeded onto FN-coated wells of 6-well culture plates with a density of 2.5 × 10⁵ cells/well. After allowing cells to spread for 30 min in an incubator, non-adherent cells were washed away with two changes of PBS. Images of adherent cells from random areas within each sample were recorded with the Nikon inverted microscope (TE300) equipped with a Hamamatsu Orca100 CCD camera.

The effect of HSPG disruption on cell adhesion strength was further assessed by cell detachment experiments using a flow chamber that generated a linear gradient of shear stress as described previously (Usami et al., 1993). In brief, ECs with or without Hep II treatment were seeded onto a FN-adsorbed glass slide (75 × 25 mm), and were incubated for 30 min in an

incubator. A tapered flow channel (0.0125 cm in height, 3.6 cm in length, and 0.22 cm in entrance width) was created by stacking the glass slide with EC culture on top of an acrylic plate with a silicon gasket with a profile of the Hele-Shaw flow streamline (Usami et al., 1993). A laminar flow with maximum wall shear stress of 2,500 dyn/cm² was delivered with a syringe pump (Cole-Parmer Instrument Company) for 10 sec. The flow across the tapered flow channel generated a linear gradient of the wall shear stress along the centerline of the channel. Shear stress at each sampling area was calculated with this equation: $\tau = (6\mu Q/Wh^2)(1 - z/L)$, where τ is shear stress, μ is viscosity of the medium, Q is the flow rate across the flow chamber, z is the distance from the inlet, and L is the length of the flow chamber. Using a Nikon inverted microscope (TE300) equipped with a Hamamatsu Orca100 CCD camera, the phase-contrast images in each area along the centerline of the chamber were recorded before and after the application of shear stress for 10 sec. The cell numbers were quantified from these images, and the percentage of cells remaining in each area was calculated.

Time-lapse confocal microscopy of GFP-tagged paxillin

GFP-paxillin was expressed in BAECs to monitor the molecular dynamics of FAs. DNA plasmids encoding GFP-paxillin was a gift from Dr. Christopher Turner at SUNY Health Science Center at Syracuse. The DNA plasmids were purified with QIAGEN Plasmid Maxi Kits (QIAGEN, Inc., Valencia, CA). DNA plasmids encoding GFP-paxillin were transfected into BAECs by using LipofectAmine PLUS reagent (from GibcoBRL) according to the instructions from the manufacturer. This method resulted in ~40% transfection efficiency in BAECs. After incubation for 5 h, the transfected cells were washed with PBS and maintained in the complete medium to reach confluence. Two days post-transfection, the transfected ECs were detached with trypsin, resuspended, and incubated in low-serum medium with or without Hep II, and seeded on Lab-Tek chamber coverglasses coated with FN for the experiments.

Two hours after cell adhesion, the chamber coverglass was immobilized on the stage of a Leica confocal microscope. A temperature hood built around the microscope kept the temperature at 37°C during experiments. The dynamics of GFP-paxillin in BAECs was tracked every 30 min by confocal fluorescence microscopy using a 40× oil objective. GFP was excited at a wavelength of 488 nm and detected within a band between 506 and 538 nm.

Fluorescence staining and confocal microscopy

For the staining of cytoskeleton and FAs, the cells were fixed in 4% paraformaldehyde in PBS for 15 min, followed by permeabilization with 0.5% Triton X-100 in PBS for 10 min. For actin staining, the specimens were stained with Rhodamine-conjugated phalloidin (5 U/ml, Molecular Probes) for 1 h. The images of actin structure were collected as Z-series sections with the Leica confocal microscopy system. Multiple sections (0.3 μm thick for each section) were projected onto one plane for presentation. For immunostaining of FAs, specimens were incubated with a primary antibody against vinculin (Sigma-Aldrich) or phosphorylated FAK at tyrosine 397 residue (p-FAK(Y397)) (Transduction Laboratories, Lexington, KY) for 2 h and with FITC-conjugated secondary antibody (Jackson ImmunoResearch, West Grove, PA) for 1 h, followed by confocal microscopy.

For the staining of HSPGs and chondroitin sulfate proteoglycans (CSPGs), BAECs were fixed for 15 min in PBS containing 4% paraformaldehyde, 1.5 mM CaCl₂, and 0.1% glutaraldehyde. The cells were incubated with the monoclonal antibody against heparan sulfate (Seikagaku Corp., Japan) or CSPGs (Chemicon Corp, Temecula, CA) for 1 h, and with FITC-conjugated secondary antibody (Jackson ImmunoResearch) for 1 h, followed by confocal microscopy.

RESULTS

Hep II specifically disrupted HSPGs

To demonstrate that Hep II specifically disrupted HSPGs in BAECs, the cells with or without Hep II

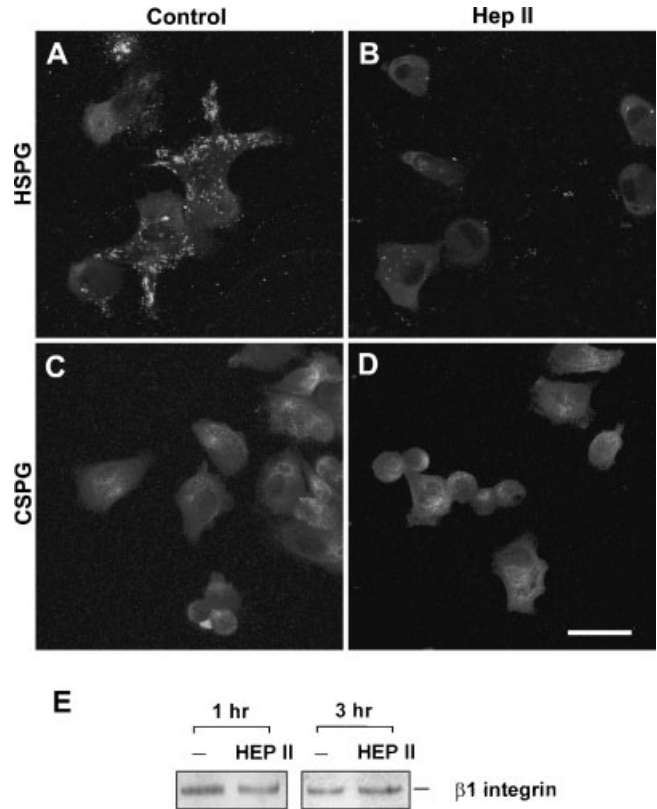


Fig. 1. Effects of Heparinase II (Hep II) on cell surface molecules. Bovine aortic endothelial cells (BAECs) with or without pretreatment of Hep II were plated onto FN-coated glass slides for 1–3 h. **A–D**: After 1 h adhesion, the cells were fixed and stained on heparan sulfate or chondroitin sulfate proteoglycans (CSPGs), and observed by confocal microscopy. Bar = 30 μm. **E**: After 1 or 3 h adhesion, the cells were lysed, and equal amount of proteins from each sample were used for immunoblotting analysis for β1 integrin expression.

treatment were subjected to immunostaining and immunoblotting analysis. As shown in Figure 1, Hep II treatment decreased cell surface heparan sulfate and cell spreading, but did not affect the cell surface CSPGs. In addition, Hep II did not affect the expression of β1 integrin—a major integrin receptor for FN.

Disruption of HSPGs by Hep II decreased EC adhesion rate and strength

To determine the role of HSPG on EC adhesion, ECs with or without pre-treatment of Hep II were allowed to spread on FN-coated wells for 30 min, and non-adherent cells were washed away with PBS. Disruption of HSPG significantly decreased cell adhesion rate to ~60% of that for un-treated cells (Fig. 2). Cell spreading was also decreased by Hep II. Some heparinase-treated cells still remained attached, possibly through integrin-FN binding.

To further determine the contribution of HSPG on cell adhesion strength, we measured the reduction in adhesion force due to heparinase treatment. Using a syringe pump to drive flow through a tapered flow chamber, a linear shear stress gradient with maximum shear stress of 2,500 dyn/cm² was created. After 30-min adhesion subconfluent ECs were subjected to this shear stress gradient for 10 sec. The number of cells that remained adherent on the surface correlated inversely and linearly with the magnitude of shear stress (Fig. 3). Adhesion force at which 50% cell detachment occurred (F_{50%}) was extrapolated from the best-fit linear curve.

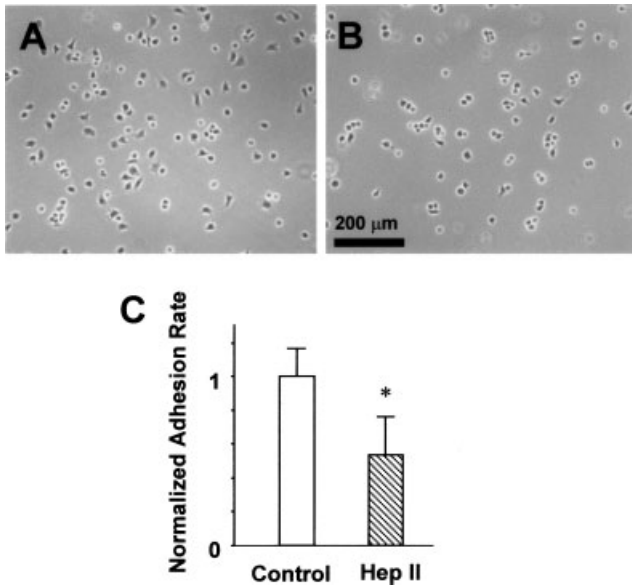


Fig. 2. Role of heparan sulfate proteoglycan (HSPG) on BAEC adhesion rate. BAECs were incubated in suspension for 30 min in DMEM with or without Hep II ($0.875 \text{ U}/10^6$ cells), and plated in FN-coated culture wells and incubated for 30 min at 37°C . After washing off non-adherent cells, phase contrast images of remaining adherent cells were taken at random areas, and cell numbers were counted. **A:** A representative phase-contrast image of BAECs without pre-treatment of Hep II. **B:** A representative phase-contrast image of BAECs with pre-treatment of Hep II. **C:** Statistical analysis of the number of adherent BAECs with or without Hep II treatment. Bars represent mean \pm standard error of mean (SEM). The number of adherent cells for the Hep II-treated samples was normalized with that of the no-treatment sample (control). * $P=0.05$ (t -test) in comparison with control.

$F_{50\%}$ for control was $2,670 \text{ dyn}/\text{cm}^2$ while $F_{50\%}$ for heparinase-treated ECs was decreased to $1,766 \text{ dyn}/\text{cm}^2$. The reduction in EC adhesion strength and the decreased cell adhesion rate (Fig. 2) due to heparinase-treatment suggest that binding of HSPG to FN significantly contributes ($\sim 33\%$) to EC attachment to the extracellular matrix.

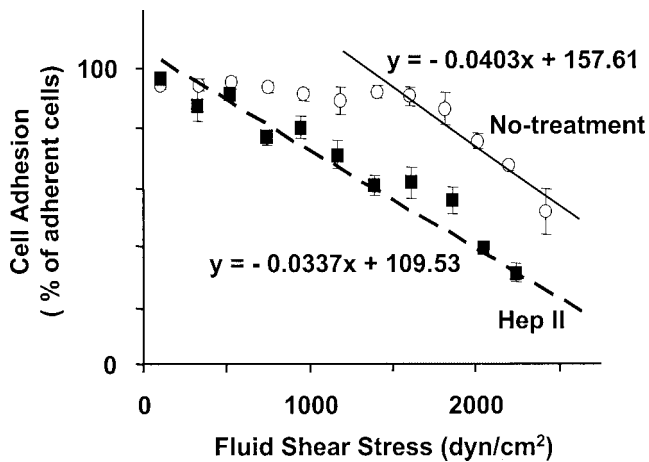


Fig. 3. Adhesion strength contributed by HSPG. Reduction in adhesion strength due to Hep II treatment was measured by applying a linear gradient of shear stress for 10 sec to subconfluent BAECs as described in "Materials and Methods." Images before and after application of shear stress were recorded along the centerline of the flow chamber. After cell counting, the percentage of remaining adherent cells after application of shear stress in each field were calculated and plotted. \circ , control; \blacksquare , Hep II-treatment. Best-fitted linear curves were plotted for the linear region of each sample. Results are presented as mean \pm SEM.

Disruption of HSPGs decreased the number of actin stress fibers and the size of FAs, and enhanced EC migration

To determine the role of HSPGs in the regulation of actin structure and FAs, ECs with or without pre-treatment of Hep II were seeded onto FN-coated glass slides for 2 h and double-stained for actin filaments and vinculin (Fig. 4). Heparinase treatment decreased stress fibers in ECs, and induced more filopodia (indicated by arrowheads in Fig. 4B). Consistently, untreated ECs had more mature and large FAs (indicated by arrows in Fig. 4C), while FAs in heparinase-treated ECs were smaller. These effects of HSPG disruption on the molecular structure of actin and FAs explain the decrease in EC adhesion rate and strength after heparinase treatment.

To determine the role of HSPG in the regulation of FA dynamics and EC migration, living ECs expressing GFP-paxillin were imaged by time-lapse confocal microscopy. Three representative cells under different conditions are shown in Figure 5. The left column is the time-lapse fluorescent microscopy of GFP-paxillin in an EC without heparinase treatment. The cell formed new FAs on the left. However, the migration was limited by the slow detachment of FAs on the right (indicated by an arrow at 30 min). In contrast, the cell pre-treated with Hep II (the middle column) had less prominent FAs. The FAs at the rear (indicated by an arrow) detached easily, which allowed efficient cell migration. To monitor the effect of HSPG disruption onto FA dynamics in the same cell, ECs were seeded on FN-coated chamber cover-glasses, and GFP-paxillin was imaged before and after

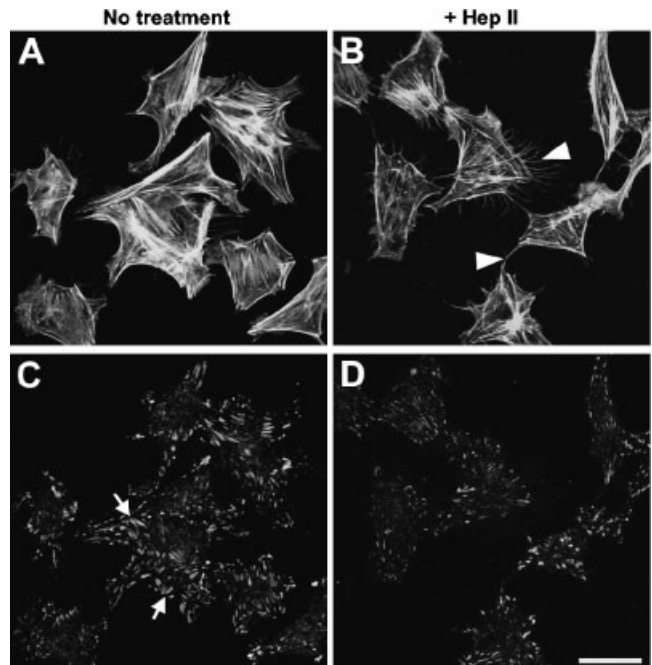


Fig. 4. Role of HSPG in actin assembly and FA formation. BAECs were incubated in suspension for 30 min in DMEM with or without Hep II, and plated onto FN-coated coverglasses and incubated for 2 h. The cells were fixed and double stained for actin and vinculin as described in "Materials and Methods." **A:** Actin filaments in endothelial cells (ECs) without treatment. **B:** Actin filaments in ECs with pre-treatment of Hep II. **C:** Vinculin staining in ECs without treatment. **D:** Vinculin staining in ECs with pre-treatment of Hep II. Bar = $30 \mu\text{m}$. The arrowheads indicate the filopodia. The arrows indicate large FAs.

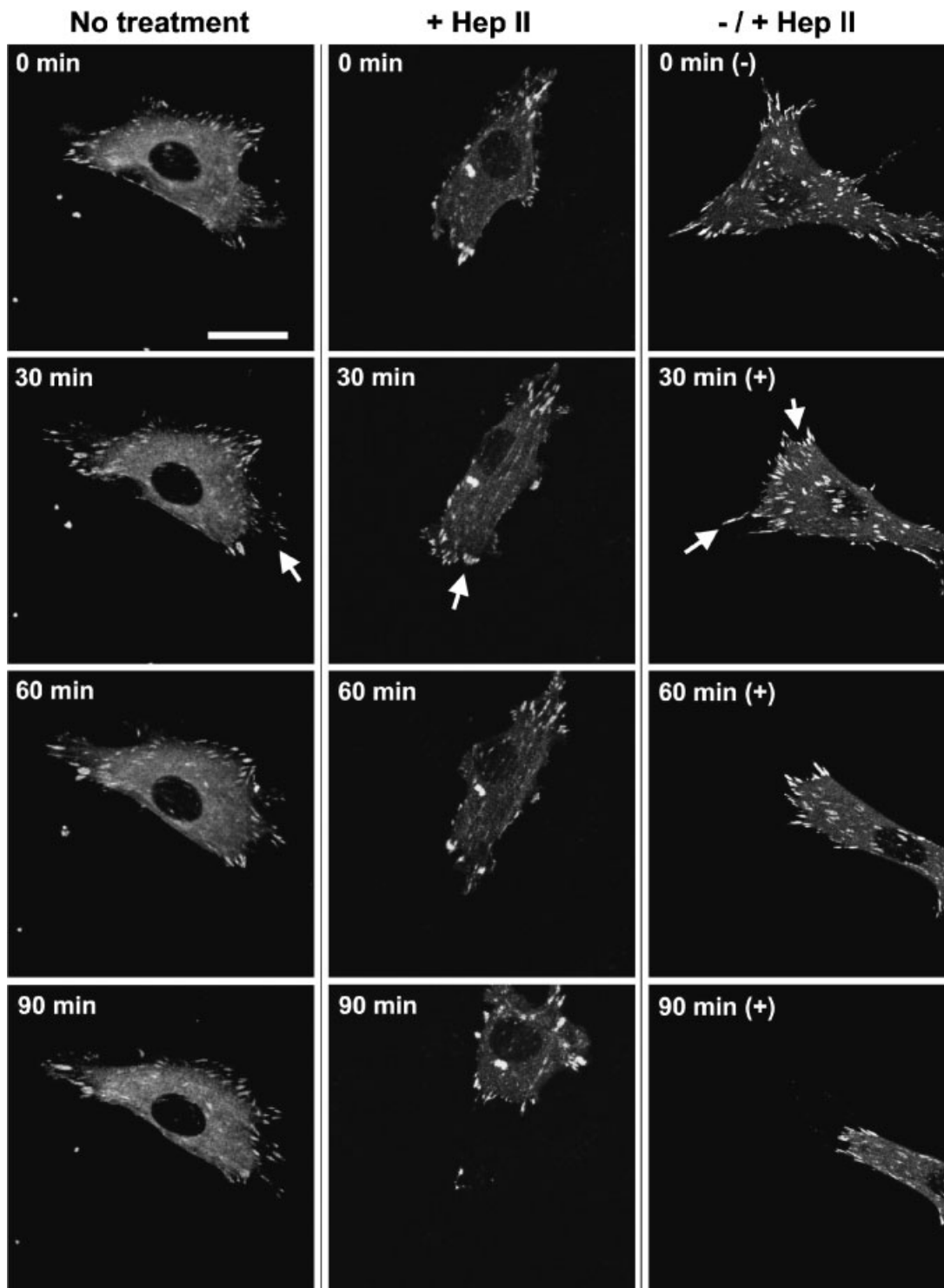


Fig. 5. Effect of HSPG disruption on FA dynamics and EC migration. ECs expressing GFP-paxillin were incubated in suspension for 30 min in the absence (columns 1 and 3) or presence (column 2) of Hep II, and seeded onto chamber coverglasses for 2 h. The fluorescence images of GFP-paxillin in selected cells were collected by using a confocal microscope at different time points. In column 3, Hep II was added to the adherent cells at 5 min time point. Bar = 20 μ m. The arrows indicate the detaching FAs.

heparinase treatment. As shown in the right column in Figure 5, the cell had prominent FAs and fully spread before Heparinase treatment ($t = 0$). After 5 min, Hep II was added to the cells, which enhanced the detachment of FAs and cell migration.

These results on actin structure and FA dynamics suggest that HSPGs promote stable adhesions and retard EC migration, and that HSPG disruption decreases stress fibers and facilitates the detachment of FAs, thus enhancing EC migration.

Disruption of HSPGs increased EC migration speed but inhibited shear stress-induced directional migration

Since ECs are constantly subjected to fluid shear stress *in vivo*, we determined the role of HSPG in EC migration under both static and flow conditions by time-lapse microscopy. Cell tracing and cell migration paths under static and flow conditions are shown in Figure 6. Phase-contrast images (Fig. 6A–D) were collected at 20-min interval for 5 h, and the centroid positions of each cell were connected at different time points to generate migration paths (Fig. 6E,F). In static culture, cell migration was random (Fig. 6A,C,E). Under flow, the cells either migrated in the flow direction or gradually turned into the flow direction (Fig. 6B,D,F).

To determine the role of HSPG in EC migration under static and flow conditions, ECs with or without pretreatment of Hep II were seeded on FN-coated glass slides, and EC migration was monitored as described in Figure 6. The migration paths of 40–50 cells under each condition were plotted with same origin in Figure 7. Under static condition, EC migration was random, and

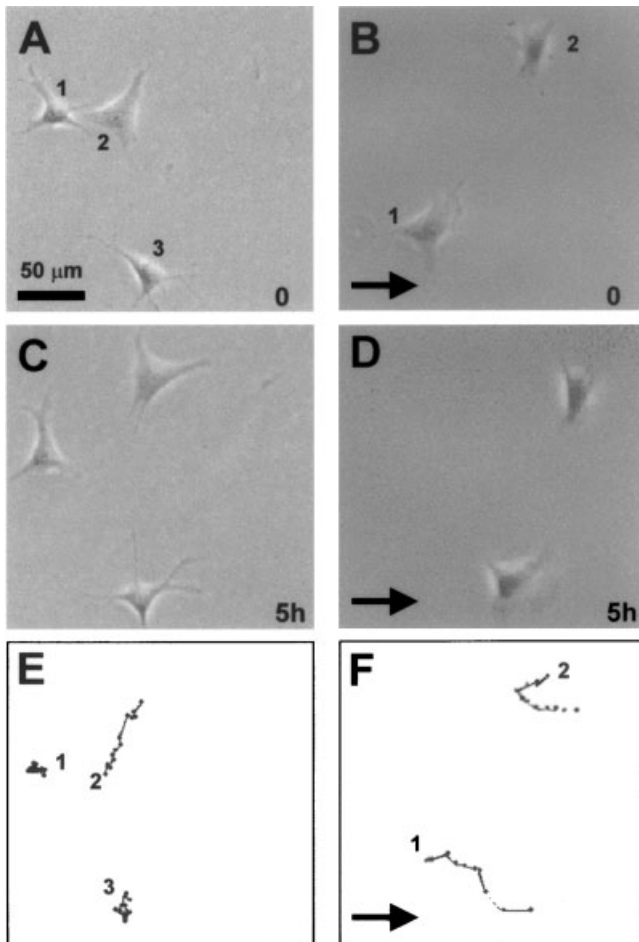


Fig. 6. Analysis of EC migration under static and flow conditions. BAECs were seeded on FN-coated slides, and were either kept as static control or subjected to fluid shear stress for 5 h. EC migration was monitored by phase-contrast microscopy at 20 min interval. Representative images of migrating cells at $t = 0$ h and 5 h under static condition (A and C) and under flow condition (B and D) are shown. Centroids of cells shown in A–D were determined with DIAS software, and the cell migration paths were generated and shown in E (static) and F (under flow), respectively. Arrows indicate the direction of shear stress. Bar = 50 μm .

the cell travel distance was dramatically increased after heparinase treatment (Fig. 7A,B), consistent with the result in Figure 5. Fluid shear stress induced directional migration of ECs, and some cells that originally migrated against flow gradually turned around (Fig. 7C). In contrast, after HSPG disruption, some migration paths of heparinase-treated cells were either perpendicular or opposite to the direction of the flow (Fig. 7D). Within a 5 h period, shear stress failed to reverse the cell migration against the flow, although shear stress enhanced the migration of some cells that originally migrated in the flow direction.

To quantify the phenomenon in Figure 7, EC migration speed and direction were calculated. As shown in Figure 8A, heparinase-treated ECs migrated faster under both static and flow conditions. Under flow condition, the speed of cell migration was slightly enhanced by shear stress. These results suggest that HSPG disruption may decrease cell adhesion to the matrix and that shear stress may further augment the cell–matrix dissociation process.

The direction of EC migration was quantified with $\cos\theta$ where θ is the angle between the migration direction and flow direction (or a specified direction for static condition). As shown in Figure 8B, heparinase treatment did not affect the directionality of EC migration under static condition. In contrast, the directional migration induced by shear stress was inhibited in heparinase-treated cells. This result suggests that HSPG on the cell surface may function as a mechanotransducer to relay extracellular mechanical stimulus to intracellular mechano-chemical transduction pathways and that ECs without HSPG on their surfaces might lose their ability to sense the mechanical stimulus and migrate in the flow direction.

Decreasing cell adhesion by lowering FN density enhanced EC migration but did not affect the directional migration of ECs under flow

A possible mechanism for the loss of the directional migration of ECs under flow after HSPG disruption was that HSPG disruption decreased cell adhesion and thus decreased the EC sensitivity to flow. To determine whether the decrease of cell–matrix interaction accounted for the loss of directional migration of ECs under flow, BAECs were cultured on slides coated with lower density (0.2 $\mu\text{g}/\text{cm}^2$) of FN, on which less cell spreading was observed when compared with the cells on higher density (1 $\mu\text{g}/\text{cm}^2$) of FN (Fig. 9A,B). As shown in Figure 9C, the decrease of cell adhesion slightly enhanced EC migration under both static and flow conditions, similar to the effect of HSPG disruption. However, the decrease of cell adhesion did not affect the directional migration of ECs under flow (Fig. 9D), suggesting that the decrease of cell adhesion does not account for the loss of directional migration under flow after HSPG disruption.

Disruption of HSPGs decreased shear stress-induced FA formation and the recruitment of phosphorylated FAK in the flow direction

FAK is a cytoplasmic tyrosine kinase that localizes at FAs and mediates integrin-initiated signaling (Cary et al., 1999; Schlaepfer et al., 1999). Activation and autophosphorylation of FAK at Y397 upon cell adhesion allows FAK to associate with Src, which triggers downstream signaling events such as phosphorylation of MAPKs, p130^{cas} (CAS), and paxillin to mediate cell adhesion and migration (Burrige et al., 1992; Chen

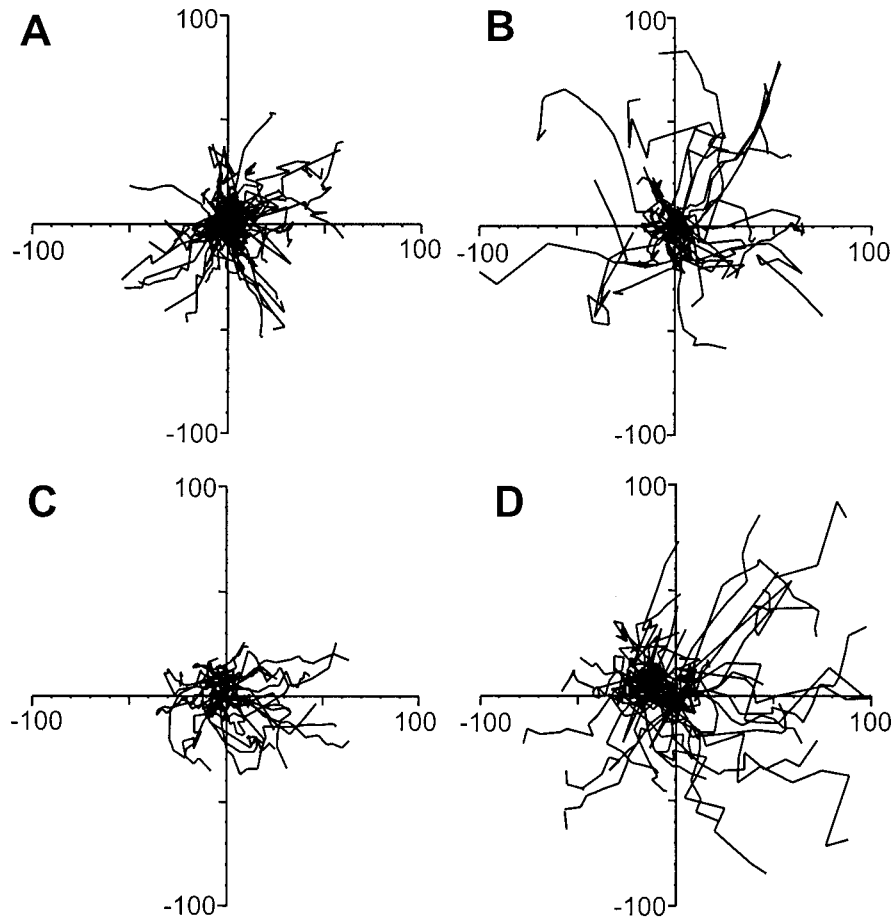


Fig. 7. Effect of HSPG disruption on EC migration under static and flow conditions. BAECs with or without heparinase-treatment were plated onto FN-coated slides. EC migration under static and flow conditions was monitored for 5 h, and the migration paths were generated as in Figure 6. Under each condition, the migration paths of 40–50 cells were plotted with same origin. **A:** Cell migration paths

under static condition without pre-treatment of Hep II. **B:** Cell migration paths under static condition after pre-treatment of Hep II. **C:** Cell migration paths under flow condition without pre-treatment of Hep II. **D:** Cell migration paths under flow condition after pre-treatment of Hep II.

et al., 1994; Schlaepfer et al., 1994; Vuori and Ruoslahti, 1995; Zhu and Assoian, 1995; Cary et al., 1998). To determine the effects of HSPG disruption on FAK activation, ECs with or without heparinase treatment were subjected to shear stress or kept as static control, and stained for p-FAK(Y397). As shown in Figure 10, heparinase treatment decreased prominent p-FAK(Y397) at FAs (Fig. 10A,B) under static condition, consistent with the results on FAs in Figures 4 and 5. Shear stress induced polarized cell morphology and p-FAK(Y397) at lamellipodia in the flow direction (indicated by arrowheads in Fig. 10C), whereas heparinase treatment decreased cell alignment, lamellipodial protrusion and its associated p-FAK(Y397) in the flow direction (Fig. 10D). These results suggest that HSPG disruption decreases shear stress-induced signaling at FAs, especially at lamellipodia in the flow direction.

DISCUSSION

We have shown that disruption of HSPGs with heparinase decreased EC adhesion, stress fibers, and the size of FAs in ECs (Figs. 2–4), which is consistent with the role of HSPGs in cell–FN interaction. FN has multiple binding domains for integrins and cell surface proteoglycans (Ruoslahti and Pierschbacher, 1987; Mohri, 1997; Romberger, 1997; Ruoslahti and Engvall, 1997; Magnusson and Mosher, 1998; Sharma et al.,

1999). FN primarily interacts with integrin $\beta 1$ or $\alpha v \beta 3$ through RGD tri-peptide in the III₁₀ region. In addition to FN–integrin interaction, FN binds to HSPGs at the cell surface via motifs in repeats FN12–14, which act in concert with FN–integrin binding to stimulate the formation of focal adhesions (Beyth and Culp, 1984; Woods et al., 1986, 1993). It has been shown that FN fragments lacking the heparin binding domain attenuates cell attachment, spreading, and cell survival in fibroblasts (Woods et al., 1986; Jeong et al., 2001).

For the first time, we quantified the contribution of HSPGs to cell adhesion strength. Our results showed that HSPGs contribute to ~33% of the adhesion strength in ECs (Fig. 3), suggesting that HSPGs play a significant role in cell–FN interaction. In the cell detachment experiments, we used very high shear stress (up to 2,500 dyn/cm²) for a short period (10 sec). By applying shear stress to cells for 10 sec, we aimed to capture the instantaneous adhesion strength, and to avoid the cellular responses such as the remodeling of actin structure and FAs and the re-enforcement of cell adhesions induced by shear stress (Li et al., 1999, 2002; Koo et al., 2002; Mathur et al., 2003).

Several HSPGs involved in FN binding have been characterized. So far two gene families of cell surface HSPGs are identified: syndecan and glypican family with four and six gene products in mammals, respec-

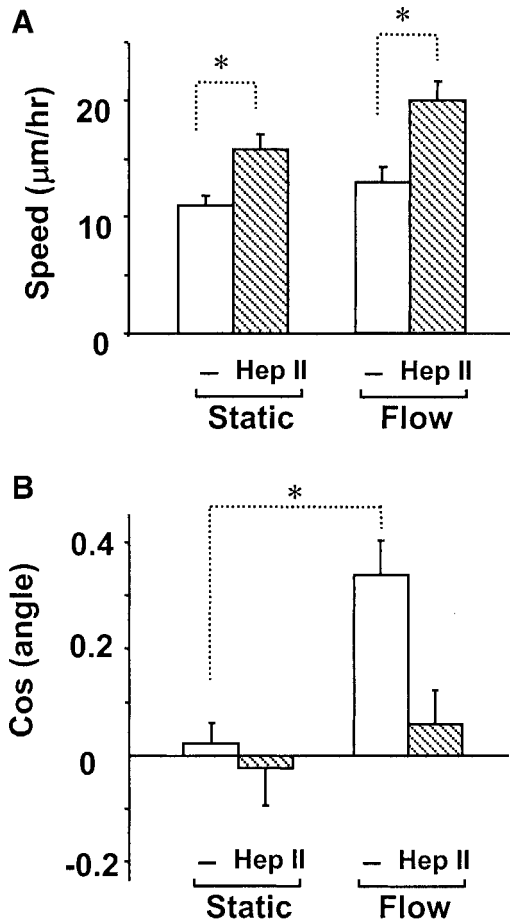


Fig. 8. Role of HSPG in shear stress-induced directional migration of ECs. BAECs with or without heparinase-treatment were plated onto FN-coated slides. EC migration under static and flow conditions was monitored for 5 h, and the migration speed and direction were calculated from migration paths using the DIAS software. More than 30 cells from each sample were included in statistical analysis. **A**: Migration speed. **B**: Migration direction. The cell migration direction was indexed with $\text{Cos}\theta$, where θ is the angle between the migration direction and flow direction. Bars represent mean \pm SEM. * $P = 0.05$ (t test).

tively (Fransson et al., 1986; Yamashita et al., 1999). Syndecans are composed of an extracellular domain, a transmembrane domain, and a short cytoplasmic domain that is capable of interacting with actin cytoskeleton and mediating signal cascades (Rapraeger, 1993; Bernfield et al., 1999; Couchman and Woods, 1999). Glypicans have an extracellular domain that is covalently linked to plasma membrane lipid by glycosylphosphatidylinositol anchor (Bernfield et al., 1999; De Cat and David, 2001). Cell surface HSPGs expressed in ECs include syndecan-1, syndecan-4, glypican-1, and glypican-4, in addition to betaglycan and CD-44 that may have HS sidechains (Mertens et al., 1992; Karumanchi et al., 2001; Jarvelainen and Wight, 2002; Iivanainen et al., 2003). Among these HSPGs, syndecan-4 plays a crucial role in focal adhesion assembly and cytoskeleton reorganization (LeBaron et al., 1988; Woods et al., 1993; Woods and Couchman, 1994, 1998; Yoneda et al., 1995; Couchman and Woods, 1999; Longley et al., 1999; Saoncella et al., 1999). However, syndecan-4 knock-out fibroblasts still form FAs and stress fibers (Ishiguro et al., 2000), suggesting that other heparin-binding receptors may also be involved in cell-matrix interactions.

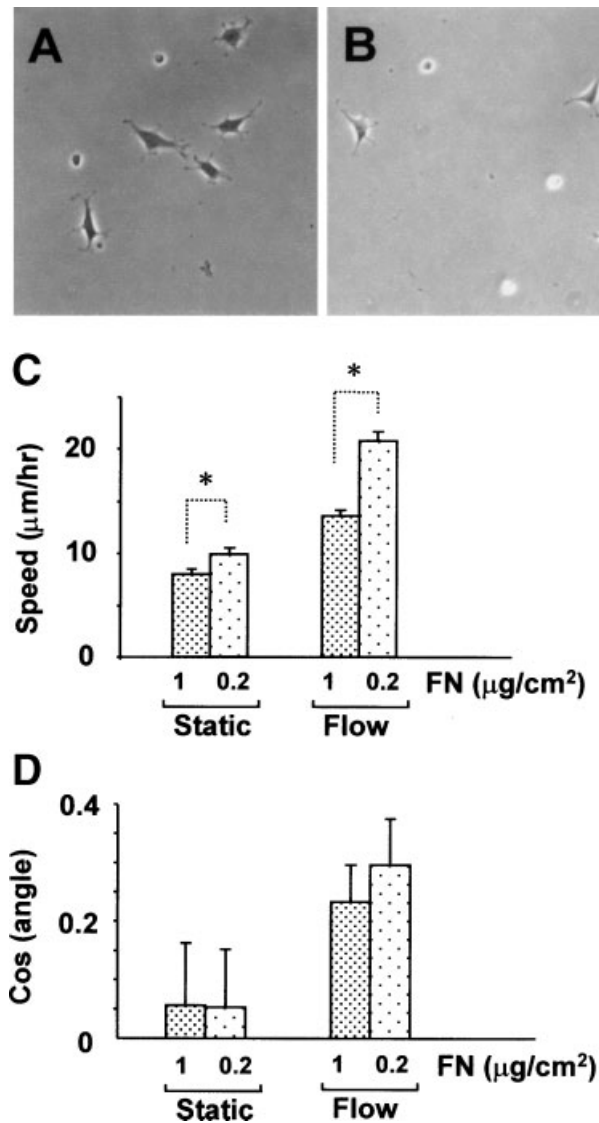


Fig. 9. Effect of decreasing cell adhesion on shear stress-induced directional migration of ECs. BAECs were plated onto slides coated with 1 or 0.2 $\mu\text{g}/\text{cm}^2$ of FN. EC migration under static and flow conditions was monitored for 5 h, and the migration speed and direction were calculated from migration paths using the DIAS software. More than 30 cells from each sample were included in statistical analysis. **A**: Phase contrast image of BAECs on 1 $\mu\text{g}/\text{cm}^2$ of FN. **B**: Phase contrast image of BAECs on 0.2 $\mu\text{g}/\text{cm}^2$ of FN. **C**: Migration speed. **D**: Migration direction. The cell migration direction was indexed with $\text{Cos}\theta$, where θ is the angle between the migration direction and flow direction. Bars represent mean \pm SEM. * $P = 0.05$ (t test).

Our results showed that heparinase treatment increased EC migration speed under both static and flow conditions (Figs. 5–8), suggesting that HSPGs promote stable FAs that may retard cell migration. Indeed, HSPG disruption decreases the size of prominent FAs (Fig. 4) and p-FAK(Y397) (Fig. 10). Interestingly, the increase of cell migration speed was more pronounced in heparinase-treated ECs than in untreated ECs under flow condition, which may be due to the increased dissociation of FAs by shear stress. These results are in agreement with a previous report that CHO cells overexpressing syndecan-4 show decreased migration and wound healing (Longley et al., 1999). However, in vivo experiments have shown that skin wound repair and

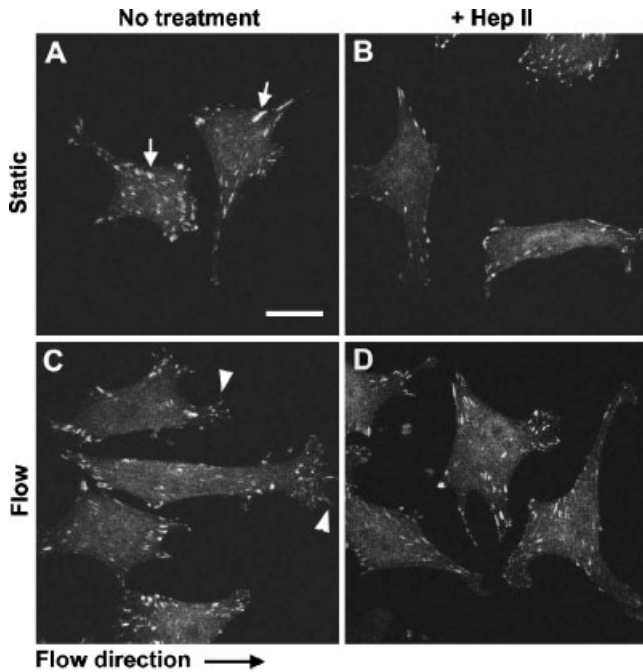


Fig. 10. Effect of HSPG disruption on FAK phosphorylation at FAs. BAECs with (in **B** and **D**) or without heparinase-treatment (in **A** and **C**) were plated onto FN-coated slides. After 2 h incubation, the cells were either kept as static control (in **A** and **B**) or subjected to shear stress (in **C** and **D**) for 2 h. The cells were fixed and stained with an antibody against phospho-FAK(Y397). Bar = 25 μ m. The arrows in **A** indicate prominent FAs in cells. The arrowheads in **C** indicate new FAs formed at the leading edge in the flow direction.

angiogenesis are impaired by the disruption of syndecan-4 gene (Echtermeyer et al., 2001). One explanation for this discrepancy is that HSPGs have multiple functions, e.g., mediation of growth factor bindings

and interactions with many matrix proteins, and that the HSPG disruption may impair these functions thus indirectly affecting cell migration.

In addition to regulating cell adhesion and migration speed, HSPGs seem to be important in sensing the direction of shear stress. Upon exposure to shear stress, ECs are known to polarize their cell body and reorient their path of migration to the direction of shear stress (Li et al., 2002). However, this mechanotaxis process is drastically attenuated in ECs with disrupted cell surface HSPGs (Figs. 7–8), and the recruitment and activation of FAK at lamellipodia in the flow direction are reduced (Fig. 10). Since the decrease of EC adhesion by lowering the FN coating density did not inhibit the directional EC migration induced by shear stress (Fig. 9), the inhibition of mechanotaxis by HSPG disruption is unlikely due to the decrease of cell adhesions at the abluminal surface of ECs. Rather it may be related to the HSPGs on the luminal surface. The extensive polysaccharide structure of HSPGs sticking out on the luminal surface of ECs may function as sensors and transducers of shear stress, and transmits the extracellular stimulation via the interaction of HSPGs with plasma membranes and underlying actin structure. A recent theoretical analysis predicts that the glycocalyx can deform in response to shear stress and transmit the bending and torque into cells (Weinbaum et al., 2003). Furthermore, recent studies have shown that the disruption of hyaluronic acid glycosaminoglycans or HSPG in the glycocalyx attenuates shear stress-induced nitric oxide release (Florjan et al., 2003; Mochizuki et al., 2003). Based on these results, we propose that HSPGs play dual roles as mechanotransducer on the EC surface (Fig. 11): (1) HSPGs–matrix interaction on the abluminal surface regulates EC migration speed through an adhesion-dependent manner, and (2) HSPGs that are not binding to matrix (e.g., on the luminal surface) are involved in sensing the direction of flow through an adhesion-independent manner.

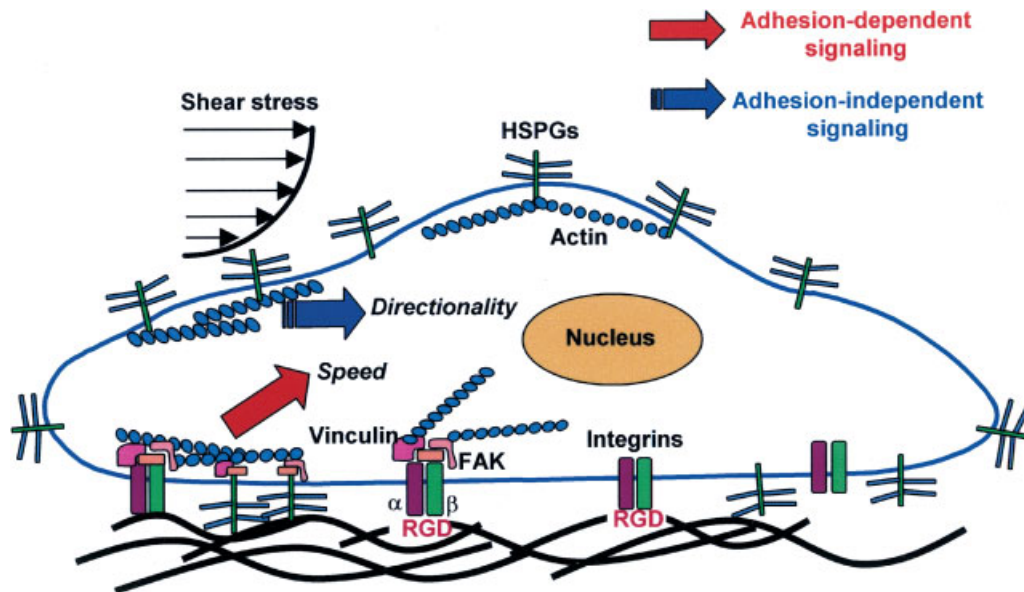


Fig. 11. A model on the dual roles of HSPGs in mechanotransduction during EC migration. ECs adhere to matrix proteins through adhesion receptors such as integrins and HSPGs. The cytoplasmic domains of integrins and HSPGs link to actin cytoskeleton through proteins in focal complexes (e.g., vinculin, FAK). The adhesion-dependent signaling can regulate EC migration speed. On the other hand, HSPGs

without binding to matrix proteins (e.g., on the luminal surface) are involved in sensing the direction of shear stress and transmitting the mechanical signal into intracellular space through cell membrane or underlying actin cytoskeleton. This adhesion-independent signaling regulates the direction of EC migration.

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LITERATURE CITED

- Albuquerque ML, Flozak AS. 2001. Patterns of living β -actin movement in wounded human coronary artery endothelial cells exposed to shear stress. *Exp Cell Res* 270(2):223–234.
- Albuquerque ML, Waters CM, Savla U, Schnaper HW, Flozak AS. 2000. Shear stress enhances human endothelial cell wound closure in vitro. *Am J Physiol Heart Circ Physiol* 279(1):H293–H302.
- Ando J, Nomura H, Kamiya A. 1987. The effect of fluid shear stress on the migration and proliferation of cultured endothelial cells. *Microvasc Res* 33(1):62–70.
- Bernfield M, Gotte M, Park PW, Reizes O, Fitzgerald ML, Lincecum J, Zako M. 1999. Functions of cell surface heparan sulfate proteoglycans. *Annu Rev Biochem* 68:729–777.
- Beyth RJ, Culp LA. 1984. Complementary adhesive responses of human skin fibroblasts to the cell-binding domain of fibronectin and the heparan sulfate-binding protein, platelet factor-4. *Exp Cell Res* 155(2):537–548.
- Boardman KC, Swartz MA. 2003. Interstitial flow as a guide for lymphangiogenesis. *Circ Res* 92(7):801–808.
- Branemark PI. 1965. Capillary form and function. The microcirculation of granulation tissue. *Bibl Anat* 7:9–28.
- Burridge K, Turner CE, Romer LH. 1992. Tyrosine phosphorylation of paxillin and pp125FAK accompanies cell adhesion to extracellular matrix: A role in cytoskeletal assembly. *J Cell Biol* 119(4):893–903.
- Cary LA, Han DC, Polte TR, Hanks SK, Guan JL. 1998. Identification of p130Cas as a mediator of focal adhesion kinase-promoted cell migration. *J Cell Biol* 140(1):211–221.
- Cary LA, Han DC, Guan JL. 1999. Integrin-mediated signal transduction pathways. *Histol Histopathol* 14(3):1001–1009.
- Chen Q, Kinch MS, Lin TH, Burridge K, Juliano RL. 1994. Integrin-mediated cell adhesion activates mitogen-activated protein kinases. *J Biol Chem* 269(43):26602–26605.
- Couchman JR, Woods A. 1999. Syndecan-4 and integrins: Combinatorial signaling in cell adhesion. *J Cell Sci* 112(Pt 20):3415–3420.
- De Cat B, David G. 2001. Developmental roles of the glypicans. *Semin Cell Dev Biol* 12(2):117–125.
- Echtermeyer F, Streit M, Wilcox-Adelman S, Saoncella S, Denhez F, Detmar M, Goetinck P. 2001. Delayed wound repair and impaired angiogenesis in mice lacking syndecan-4. *J Clin Invest* 107(2):R9–R14.
- Florian JA, Kosky JR, Ainslie K, Pang Z, Dull RO, Tarbell JM. 2003. Heparan sulfate proteoglycan is a mechanosensor on endothelial cells. *Circ Res* 93(10):e136–e42.
- Fransson LA, Carlstedt I, Coster L, Malmstrom A. 1986. The functions of the heparan sulphate proteoglycans. *Ciba Found Symp* 124:125–142.
- Hsu PP, Li S, Li YS, Usami S, Ratcliffe A, Wang X, Chien S. 2001. Effects of flow patterns on endothelial cell migration into a zone of mechanical denudation. *Biochem Biophys Res Commun* 285(3):751–759.
- Hu YL, Li S, Miao H, Tsou TC, del Pozo MA, Chien S. 2002. Roles of microtubule dynamics and small GTPase rac in endothelial cell migration and lamellipodium formation under flow. *J Vasc Res* 39(6):465–476.
- Iivanainen E, Kahari VM, Heino J, Elenius K. 2003. Endothelial cell–matrix interactions. *Microsc Res Tech* 60(1):13–22.
- Ishiguro K, Kadomatsu K, Kojima T, Muramatsu H, Tsuzuki S, Nakamura E, Kusugami K, Saito H, Muramatsu T. 2000. Syndecan-4 deficiency impairs focal adhesion formation only under restricted conditions. *J Biol Chem* 275(8):5249–5252.
- Jarvelainen H, Wight TN. 2002. Vascular proteoglycans. In: Garg HG, Roughley PJ, Hales CA, editors. *Proteoglycans and lung disease*. New York: Marcel Dekker. pp 291–321.
- Jeong J, Han I, Lim Y, Kim J, Park I, Woods A, Couchman JR, Oh ES. 2001. Rat embryo fibroblasts require both the cell-binding and the heparin-binding domains of fibronectin for survival. *Biochem J* 356(Pt 2):531–537.
- Kapila YL, Wang S, Johnson PW. 1999. Mutations in the heparin binding domain of fibronectin in cooperation with the V region induce decreases in pp125(FAK) levels plus proteoglycan-mediated apoptosis via caspases. *J Biol Chem* 274(43):30906–30913.
- Karumanchi SA, Jha V, Ramchandran R, Karihaloo A, Tsiokas L, Chan B, Dhanabal M, Hanai JL, Venkataraman G, Shriver Z, Keiser N, Kalluri R, Zeng H, Mukhopadhyay D, Chen RL, Lander AD, Hagihara K, Yamaguchi Y, Sasisekharan R, Cantley L, Sukhatme VP. 2001. Cell surface glypicans are low-affinity endostatin receptors. *Mol Cell* 7(4):811–822.
- Koo LY, Irvine DJ, Mayes AM, Lauffenburger DA, Griffith LG. 2002. Co-regulation of cell adhesion by nanoscale RGD organization and mechanical stimulus. *J Cell Sci* 115(Pt 7):1423–1433.
- Lauffenburger DA, Horwitz AF. 1996. Cell migration: A physically integrated molecular process. *Cell* 84(3):359–369.
- LeBaron RG, Esko JD, Woods A, Johansson S, Hook M. 1988. Adhesion of glycosaminoglycan-deficient chinese hamster ovary cell mutants to fibronectin substrata. *J Cell Biol* 106(3):945–952.
- Li S, Chen BP, Azuma N, Hu YL, Wu SZ, Sumpio BE, Shyy JY, Chien S. 1999. Distinct roles for the small GTPases Cdc42 and Rho in endothelial responses to shear stress. *J Clin Invest* 103(8):1141–1150.
- Li S, Butler P, Wang Y, Hu Y, Han DC, Usami S, Guan JL, Chien S. 2002. The role of the dynamics of focal adhesion kinase in the mechanotaxis of endothelial cells. *Proc Natl Acad Sci USA* 99(6):3546–3551.
- Linhardt RJ, Turnbull JE, Wang HM, Loganathan D, Gallagher JT. 1990. Examination of the substrate specificity of heparin and heparan sulfate lyases. *Biochemistry* 29(10):2611–2617.
- Longley RL, Woods A, Fleetwood A, Cowling GJ, Gallagher JT, Couchman JR. 1999. Control of morphology, cytoskeleton and migration by syndecan-4. *J Cell Sci* 112(Pt 20):3421–3431.
- Luft JH. 1966. Fine structures of capillary and endocapillary layer as revealed by ruthenium red. *Fed Proc* 25(6):1773–1783.
- Magnusson MK, Mosher DF. 1998. Fibronectin: Structure, assembly, and cardiovascular implications. *Arterioscler Thromb Vasc Biol* 18(9):1363–1370.
- Marcum JA, Rosenberg RD. 1987. Anticoagulant active heparan sulfate proteoglycan and the vascular endothelium. *Semin Thromb Hemost* 13(4):464–474.
- Masuda M, Fujiwara K. 1993. The biased lamellipodium development and microtubule organizing center position in vascular endothelial cells migrating under the influence of fluid flow. *Biol Cell* 77(3):237–245.
- Mathur AB, Truskey GA, Reichert WM. 2003. Synergistic effect of high-affinity binding and flow preconditioning on endothelial cell adhesion. *J Biomed Mater Res* 64A(1):155–163.
- Mertens G, Cassiman JJ, Van den Berghe H, Vermylen J, David G. 1992. Cell surface heparan sulfate proteoglycans from human vascular endothelial cells. Core protein characterization and antithrombin III binding properties. *J Biol Chem* 267(28):20435–20443.
- Mochizuki S, Vink H, Hiramatsu O, Kajita T, Shigeto F, Spaan JA, Kajiya F. 2003. Role of hyaluronic acid glycosaminoglycans in shear-induced endothelium-derived nitric oxide release. *Am J Physiol Heart Circ Physiol* 285(2):H722–H726.
- Mohri H. 1997. Interaction of fibronectin with integrin receptors: Evidence by use of synthetic peptides. *Peptides* 18(6):899–907.
- Moldovan NI, Goldschmidt-Clermont PJ, Parker-Thornburg J, Shapiro SD, Kolattukudy PE. 2000. Contribution of monocytes/macrophages to compensatory neovascularization: The drilling of metalloelastase-positive tunnels in ischemic myocardium. *Circ Res* 87(5):378–384.
- Moyano JV, Carnemolla B, Albar JP, Leprini A, Gaggero B, Zardi L, Garcia-Pardo A. 1999. Cooperative role for activated $\alpha 4$ $\beta 1$ integrin and chondroitin sulfate proteoglycans in cell adhesion to the heparin III domain of fibronectin. Identification of a novel heparin and cell binding sequence in repeat III5. *J Biol Chem* 274(1):135–142.
- Rapraeger AC. 1993. The coordinated regulation of heparan sulfate, syndecans and cell behavior. *Curr Opin Cell Biol* 5(5):844–853.
- Rapraeger AC, Krufka A, Olwin BB. 1991. Requirement of heparan sulfate for bFGF-mediated fibroblast growth and myoblast differentiation. *Science* 252(5013):1705–1708.
- Romberger DJ. 1997. Fibronectin. *Int J Biochem Cell Biol* 29(7):939–943.
- Ruoslahti E, Engvall E. 1997. Integrins and vascular extracellular matrix assembly. *J Clin Invest* 100(Suppl 11):S53–S56.
- Ruoslahti E, Pierschbacher MD. 1987. New perspectives in cell adhesion: RGD and integrins. *Science* 238(4826):491–497.
- Saksela O, Moscatelli D, Sommer A, Rifkin DB. 1988. Endothelial cell-derived heparan sulfate binds basic fibroblast growth factor and protects it from proteolytic degradation. *J Cell Biol* 107(2):743–751.
- Saoncella S, Echtermeyer F, Denhez F, Nowlen JK, Mosher DF, Robinson SD, Hynes RO, Goetinck PF. 1999. Syndecan-4 signals cooperatively with integrins in a Rho-dependent manner in the assembly of focal adhesions and actin stress fibers. *Proc Natl Acad Sci USA* 96(6):2805–2810.
- Schlaepfer DD, Hanks SK, Hunter T, van der Geer P. 1994. Integrin-mediated signal transduction linked to Ras pathway by GRB2 binding to focal adhesion kinase. *Nature* 372(6508):786–791.
- Schlaepfer DD, Hauck CR, Sieg DJ. 1999. Signaling through focal adhesion kinase. *Prog Biophys Mol Biol* 71(3–4):435–478.
- Sharma A, Askari JA, Humphries MJ, Jones EY, Stuart DI. 1999. Crystal structure of a heparin- and integrin-binding segment of human fibronectin. *EMBO J* 18(6):1468–1479.
- Sheetz MP, Felsenfeld DP, Galbraith CG. 1998. Cell migration: Regulation of force on extracellular–matrix–integrin complexes. *Trends Cell Biol* 8(2):51–54.
- Sprague EA, Luo J, Palmaz JC. 1997. Human aortic endothelial cell migration onto stent surfaces under static and flow conditions. *J Vasc Interv Radiol* 8:83–92.
- Squire JM, Chew M, Nneji G, Neal C, Barry J, Michel C. 2001. Quasi-periodic substructure in the microvessel endothelial glycocalyx: A possible explanation for molecular filtering? *J Struct Biol* 136(3):239–255.
- Tanaka Y, Fujii K, Hubscher S, Aso M, Takazawa A, Saito K, Ota T, Eto S. 1998. Heparan sulfate proteoglycan on endothelium efficiently induces integrin-mediated T cell adhesion by immobilizing chemokines in patients with rheumatoid synovitis. *Arthritis Rheum* 41(8):1365–1377.
- Urbich C, Dernbach E, Reissner A, Vasa M, Zeiher AM, Dimmeler S. 2002. Shear stress-induced endothelial cell migration involves integrin signaling via the fibronectin receptor subunits $\alpha 5 \beta 1$. *Arterioscler Thromb Vasc Biol* 22(1):69–75.
- Usami S, Chen HH, Zhao Y, Chien S, Skalak R. 1993. Design and construction of a linear shear stress flow chamber. *Ann Biomed Eng* 21(1):77–83.
- van Kooten TG, Schakenraad JM, van der Mei HC, Dekker A, Kirkpatrick CJ, Busscher HJ. 1994. Fluid shear induced endothelial cell detachment from glass—Influence of adhesion time and shear stress. *Med Eng Phys* 16(6):506–512.
- Vink H, Duling BR. 1996. Identification of distinct luminal domains for macromolecules, erythrocytes, and leukocytes within mammalian capillaries. *Circ Res* 79(3):581–589.
- Vuori K, Ruoslahti E. 1995. Tyrosine phosphorylation of p130Cas and cortactin accompanies integrin-mediated cell adhesion to extracellular matrix. *J Biol Chem* 270(38):22259–22262.
- Weinbaum S, Zhang X, Han Y, Vink H, Cowin SC. 2003. Mechanotransduction and flow across the endothelial glycocalyx. *Proc Natl Acad Sci USA* 100(13):7988–7995.
- Wojciak-Stothard B, Ridley AJ. 2003. Shear stress-induced endothelial cell polarization is mediated by Rho and Rac but not Cdc42 or PI 3-kinases. *J Cell Biol* 161(2):429–439.
- Woods A, Couchman JR. 1994. Syndecan 4 heparan sulfate proteoglycan is a selectively enriched and widespread focal adhesion component. *Mol Biol Cell* 5(2):183–192.
- Woods A, Couchman JR. 1998. Syndecans: Synergistic activators of cell adhesion. *Trends Cell Biol* 8(5):189–192.
- Woods A, Couchman JR, Johansson S, Hook M. 1986. Adhesion and cytoskeletal organization of fibroblasts in response to fibronectin fragments. *EMBO J* 5(4):665–670.

- Woods A, McCarthy JB, Furcht LT, Couchman JR. 1993. A synthetic peptide from the COOH-terminal heparin-binding domain of fibronectin promotes focal adhesion formation. *Mol Biol Cell* 4(6):605–613.
- Woods A, Longley RL, Tumova S, Couchman JR. 2000. Syndecan-4 binding to the high affinity heparin-binding domain of fibronectin drives focal adhesion formation in fibroblasts. *Arch Biochem Biophys* 374(1):66–72.
- Yamashita Y, Oritani K, Miyoshi EK, Wall R, Bernfield M, Kincade PW. 1999. Syndecan-4 is expressed by B lineage lymphocytes and can transmit a signal for formation of dendritic processes. *J Immunol* 162(10):5940–5948.
- Yayon A, Klagsbrun M, Esko JD, Leder P, Ornitz DM. 1991. Cell surface, heparin-like molecules are required for binding of basic fibroblast growth factor to its high affinity receptor. *Cell* 64(4):841–848.
- Yoneda J, Saiki I, Igarashi Y, Kobayashi H, Fujii H, Ishizaki Y, Kimizuka F, Kato I, Azuma I. 1995. Role of the heparin-binding domain of chimeric peptides derived from fibronectin in cell spreading and motility. *Exp Cell Res* 217(1):169–179.
- Zhu X, Assoian RK. 1995. Integrin-dependent activation of MAP kinase: A link to shape-dependent cell proliferation. *Mol Biol Cell* 6(3):273–282.