Syndecan-4 Clustering Induces Cell Migration in a PDZ-Dependent Manner

Eugene Tkachenko,* Arye Elfenbein,* Daniela Tirziu, Michael Simons

Abstract—Cell migration is a dynamic process involving formation of a leading edge in the direction of migration and adhesion points from which tension is generated to move the cell body forward. At the same time, disassembly of adhesion points occurs at the back of the cell, a region known as the trailing edge. Syndecan-4 (S4) is a transmembrane proteoglycan thought to be involved in the formation of focal adhesions. Recent studies have shown that its cytoplasmic domain can engage in signal transduction, making S4 a bona fide receptor. Here, we show that ligand clustering of cell surface S4 on endothelial cells initiates a signaling cascade that results in activation of Rac1, induction of cell polarization, and stimulation of cell migration that depends on S4 interaction with its PDZ-binding partner. Expression of an S4 mutant lacking its PDZ-binding region (S4-PDZ$^-$) leads to decreased cell motility and a failure to form a trailing edge. On clustering S4, but not S4-PDZ$^-$, targets activated Rac1 to the leading edge of live cells. Cells lacking synectin, a PDZ domain containing protein that interacts with S4, fail to migrate in response to S4 clustering. Both S4-PDZ$^-$–expressing and synectin$^{-/-}$ endothelial cells exhibit elevated basal levels of Rac1. Thus, our data suggest that S4 promotes endothelial cell migration in response to ligand binding by activating Rac1 and localizing it to the leading edge, and that these processes are dependent on its PDZ-binding domain interaction with synectin. **(*Circ Res*. 2006;98:1398-1404.)**

Key Words: syndecan ■ synectin ■ migration ■ endothelial cells ■ Rac1 ■ PDZ

Syndecans are a family of four transmembrane proteoglycans containing both heparan sulfate and chondroitin sulfate chains. Different family members are expressed in various cell types, with syndecan-4 (S4) demonstrating nearly ubiquitous expression. Syndecans have been thought to act as coreceptors for various heparin-binding growth factors such as fibroblast growth factors (FGFs), vascular endothelial growth factors, and fibronectin-binding integrins.[1–4] However, recent studies have suggested that the intracellular domains of syndecans, and in particular, the S4 intracellular domain, can directly engage in signal transduction.[5–9]

The cytoplasmic tail of the syndecans contains two highly conserved domains. The first (C1) is the membrane-proximal region that binds tubulin, Src kinase, ezrin, and cortactin.[3] The second (C2) is a C-terminal region that contains a PDZ-domain binding motif. The part of the molecule between the two conserved domains has been termed the variable domain, and its sequence is unique to each syndecan family member. The variable domain of S4 binds to the phosphatidyl inositol 4,5,bisphosphate/protein kinase Cα (PKCα) complex, α-actinin, and syndesmos.[3] These interactions are responsible for the previously demonstrated S4 role in cytoskeleton regulation that includes formation of focal adhesions, of dynamic stress fibers, and of cell protrusions.[9–15]

S4 is an acute response molecule, highly expressed in ischemic tissues, vascular tissues after injury,[16,17] and in a variety of solid tumors.[16–18] Its potential role in postnatal angiogenesis is supported by delayed dermal wound healing observed in S4 null mice.[19] Several reports have implicated S4 as a mediator of growth factor–induced migration.[7,8,20,21] For example, mutations of either phosphatidyl inositol 4,5,bisphosphate or PDZ-interacting regions result in impaired endothelial cell migration and proliferation in response to FGF2,[7] and the presence of the S4 cytoplasmic tail is necessary for FGF2 responsiveness.[8] Similarly, regeneration of muscles in S4 null mice is impaired because of the inability of satellite cells to migrate in response to FGF2 or hepatocyte growth factor.[21]

Evidence of direct proteoglycan signaling ability also comes from studies in smooth muscle cells, in which, despite the presence of a dominant-negative FGF receptor 1, FGF2 activated extracellular signal-regulated kinase 1/2 and promoted cell migration.[22] Using a more direct approach in the investigation of S4-induced signaling that involved antibody clustering, we previously demonstrated S4-dependent activation of Rac1, a Rho-GTPase involved in cell migration, in endothelial cells,[23] whereas another study reported S4 clus-

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tering–activated cell spreading.[24] Finally, increased baseline Rac1 activity was described in $S4^{-/-}$ fibroblasts. Together, these observations suggest S4 involvement in Rac1 regulation.[14]

Because formation of the leading edge of migrating cells is known to be associated with activation of Rac1,[25,26] we set out to explore the role of S4-dependent activation of Rac1 in cell migration. We find that S4 clustering leads to Rac1 activation and cell polarization that is dependent on S4–synectin interaction.

Materials and Methods

Antibodies and Reagents

Polyclonal goat IgG against extracellular domains of S4 were purchased from Santa Cruz Biotechnology, anti–hemagglutinin (HA)-fluorescein and high-affinity Fab fragments of IgG1 from Roche, anti-Fc receptor (FcR; CD64) monoclonal antibody from Abcam, nonimmune human IgG, Cy-3–conjugated and unlabeled goat anti-human F(ab′)2 fragment and Cy-5–conjugated streptavidin from Jackson ImmunoResearch, secondary antibodies conjugated to horseradish peroxidase from Vector Laboratories, and fluorescently labeled secondary antibodies were provided by Molecular Probes.

$S4^{-/-}$ mice were a kind gift of T. Kojima (Nagoya University, Japan), and $synectin^{-/-}$ mice were generated in the laboratory.[27]

cDNA Constructs

FcR S4 (FcR-S4) chimera was described previously.[28] Mutation of PDZ-binding sites of the chimera and the full-length constructs by removal of the C-terminal alanine 202 was done using a polymerase chain reaction (PCR) 3′-end primer TCA CTC CTC TGG GTT GGA GTC A. PCR and cloning of the chimera into pCR3.1-Uni vector (Invitrogen) were done as described previously.[28] Adenoviruses were prepared by Harvard Gene Therapy Initiative. Constructs encoding cyan fluorescent protein (CFP)-p21-activated kinase (PAK)–yellow fluorescent protein (YFP; monitor of Rac1/CDC42 activity), and CFP-PAK-Rac1-YFP (monitor of Rac1 activity) were a gift from M. Matsuda (Osaka University, Japan).

Cell Culture, Transfection, and Transduction

Human umbilical vein endothelial cells (HUVECs; Cambrex Corporation) were cultured in endothelial growth medium-2 (Cambrex). Rat fat pad endothelial cells (RFPECs) were cultured as described previously.[29] One day before all experiments, cells were plated on fibronectin-coated dishes. A solution of 10 μg/mL of fibronectin in PBS was used to coat dishes for 30 minutes at room temperature. Stable expression of FcR-S4 and FcR-PDZ^{-} chimeras was done as described previously.[28] Transient transfections were performed using GeneJammer (Stratagene) according to manufacturer protocol. HUVECs were transduced with 10 multiplicities of infection of adenovirus. Murine endothelial cells from lung tissue were isolated as described previously,[30] and flow cytometry of RFPECs expressing FcR constructs was performed as reported previously.[28]

Cell "Wounding" Assay

For all experiments, cells were seeded on fibronectin-coated tissue culture dishes (Corning). Cell migration was measured by three to four independent "wounding" assays as described previously.[7]

Syndecan Clustering

Antibody clustering of FcR-S4 chimeras and FGF2 treatment were performed as described previously.[23,28] For clustering of endogenous S4, cells were starved before stimulation overnight in high-glucose DMEM containing 0.5% serum. A total of 3 μg/mL of anti–S4 antibodies (Santa Cruz Biotechnology) were then added to the starvation media.

Rho GTPase Pull-Down Assays

RFPECs were plated on fibronectin and placed in media containing 0.5% FBS for 24 hours before assay. The activity of Rac1 was determined at the indicated time points after chimera clustering. Active Rac1 was pulled down using an activation kit (Cytoskeleton). Briefly, cells were lysed and then incubated on beads with immobilized PAK–glutathione *S*-transferase (GST) for 1 hour at 4°C. After incubation, the beads were washed three times with wash buffer and subjected to SDS-PAGE, followed by transfer onto a polyvinylidene fluoride membrane. The membranes were probed with a mouse anti-Rac1 antibody (Cytoskeleton), then by a conjugated Alexa-488 goat anti-mouse secondary antibody (Molecular Probes) and analyzed on a Typhoon 9400 multiformat imager.[28]

Live fluorescent microscopy was done using the previously described microscopy system with climate control.[31] To measure cell velocity, virally transduced cells were seeded on glass-bottom dishes (MatTek) for 12 hours and then labeled by adding 3 μg/mL of anti-HA fluorescein isothiocyanate–Fab antibodies. After 15 minutes of incubation at 37°C, cells were washed with prewarmed culture media. Images were acquired every 5 minutes using a ×20 0.5-NA phase objective. Individual cell velocity was measured using the ImageJ program (NIH). In every experiment, velocities of 12 to 20 cells were analyzed. For fluorescent resonance energy transfer (FRET) experiments, a dual splitter from Optical Insights with Chroma filter set (505 dcxr; HQ 465/30m, HQ 560/55m) was used. Microscope excitation and dichroic filter for CFP (brightlight FF458) was from Semrock. For FRET experiments, ×20 0.75-NA and ×60 1.45-NA total internal reflection fluorescence (TIRF) objectives from Olympus were used. Ratio analysis was performed using ImagePro software from Media Cybernetics using a method described previously.[32] For TIRF acquisition, a 15-watt argon 488 laser from Spectra-Physics and illumination sideport from Olympus were used. For TIRF experiments, ×60 1.45-NA TIRF objective from Olympus was used. For colocalization studies, we used a ×100 1.4-NA objective from Olympus.

Imaging studies of protrusion formation were performed using cells that were serum starved in 0.5% FBS for 24 hours before assay. Epidermal growth factor (EGF) was added at a concentration of 20 ng/mL, and cells were imaged every 30 seconds.

Results

A variety of growth factors are able to bind and cluster S4. The resulting signaling events combine the elements of S4 signaling proper with the growth factors tyrosine kinase receptors signaling. Similarly, cell binding to extracellular matrix proteins combines the elements of S4 and integrin-dependent signaling. To completely isolate signaling events dependent solely on the S4 ligand binding and clustering, we used an anti–S4 extracellular domain antibody to cluster S4 on the surface of microvascular endothelial cells derived from the wild-type and $S4^{-/-}$ mice. Anti–S4 antibody clustering of wild-type endothelial cells induced a significant migratory response, whereas $S4^{-/-}$ endothelial cells showed no increased migration (Figure 1A). The magnitude of S4 clustering–induced migration was comparable to that induced by FGF2 (Figure 1A).

To further demonstrate that the observed stimulation of migration was attributable to signaling initiated by S4 cytoplasmic domain oligomerization, we used an FcR-S4 chimera construct consisting of an FcR extracellular and transmembrane domains linked to S4 cytoplasmic domain (FcR-S4)[28] stably expressed in an RFPEC line. We used RFPECs that stably expressed FcR-S1 construct[33] to compare the effect of S4 clustering on cellular migration with another member of syndecan family. The level of expression of FcR constructs

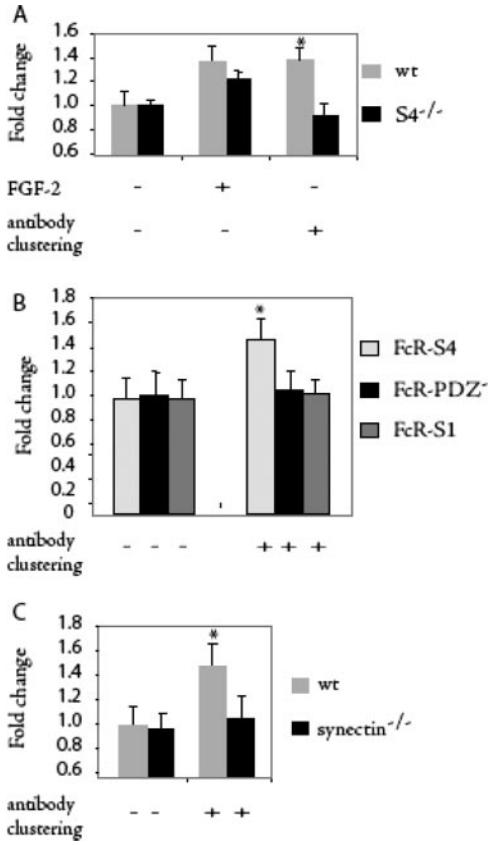

Figure 1. S4 clustering induces migration of endothelial cells in a PDZ-dependent manner. A, Primary lung endothelial cells from wild-type (wt) and S4$^{-/-}$ mice (S4$^{-/-}$) were stimulated with FGF2 or by antibody clustering of native S4. Note the migration response induced by anti-S4 antibody is similar in magnitude to that of FGF2 response in wt endothelial cells. S4$^{-/-}$ endothelial cells do not migrate in response to anti-S4 antibody (*P=0.018). B, RFPECs expressing FcR-S4, FcR-PDZ^{-}, or FcR-S1 constructs were decorated by human nonimmune IgG, followed by binding to Fab or clustering by F(ab)$_2$ portions of anti-human IgG. Note significantly enhanced migration response in FcR-S4–expressing cells (*P=0.015). C, Primary lung endothelial cells from wt and synectin$^{-/-}$ mice (synectin$^{-/-}$) were stimulated by antibody clustering of native S4. Synectin$^{-/-}$ endothelial cells do not migrate in response to anti-S4 antibody (*P=0.018). All data are shown as mean±SE of the fold change from baseline.

on the cell surface was similar for both FcR-S4 and FcR-S1 constructs (supplemental Figure I, available online at http://circres.ahajournals.org). Treatment of cells with an IgG by itself did not increase cell migration (data not shown). However, subsequent oligomerization of IgG-decorated FcR-S4–expressing cells with an F(ab)$_2$, but not F(ab), stimulated cell migration, whereas clustering of FcR-S1 did not (Figure 1B). The failure of FcR-S1 chimera–expressing cells to migrate in response to antibody clustering demonstrates the specificity of S4 transmembrane and cytoplasmic domain effects on cellular migration (Figure 1B).

To explore the role of PDZ-dependent interaction in the initiation of S4-mediated cell migration, we expressed an FcR-S4–PDZ^{-} chimera in the same RFPEC line (Figure 1B). After antibody clustering, FcR-S4 but not FcR-S4–PDZ^{-} chimera induced significant cell migration, implying that the PDZ-binding domain is required to effect S4–dependent migration (Figure 1B).

The PDZ domain-containing protein synectin was considered a potential critical partner in S4-induced migration because of its ability to bind to S4 and to mediate the migratory response of endothelial cells.[34] Antibody clustering of the native S4 (expressed equally by synectin$^{+/+}$ and synectin$^{-/-}$ endothelial cells), stimulated migration of synectin$^{+/+}$ endothelial cells to the same extent as S4-FcR clustering (Figure 1C). However, synectin$^{-/-}$ endothelial cells failed to migrate in response to S4 clustering (Figure 1C), mimicking the lack of migration seen after FcR-S4–PDZ^{-} clustering.

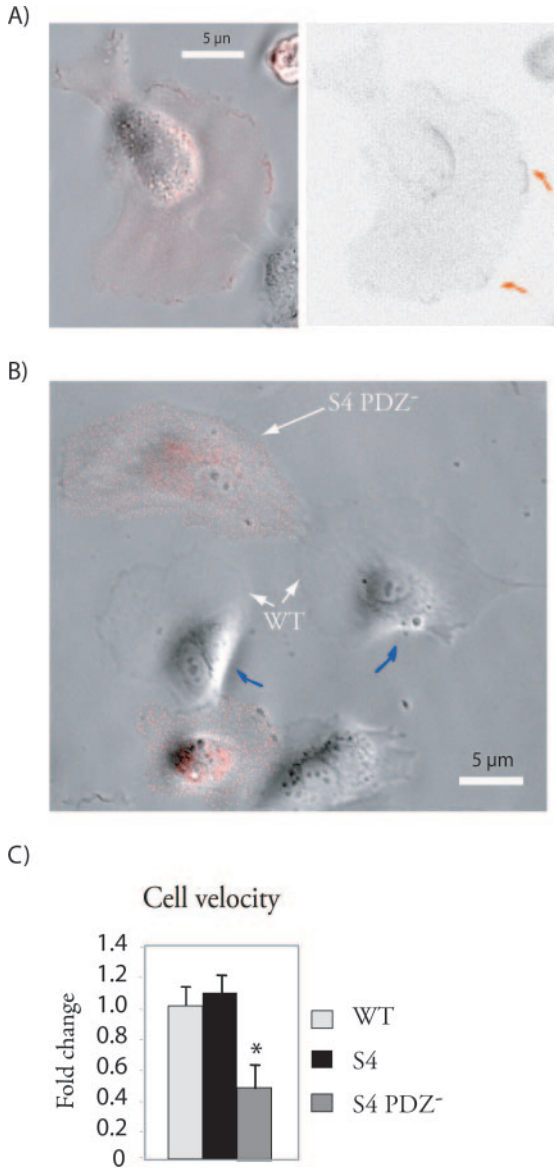

Figure 2. S4 matrix binding induces PDZ-dependent cell polarization. A, HUVECs overexpressing S4 were plated on fibronectin-coated cover slips for 3 hours. The phase image overlaid with the S4 stain (red) is shown in the left panel, and the S4-only stain is shown in the right panel only. Arrows indicate regions of S4 concentration on the lamellipodia. B, Phase contrast image of wild-type (WT; white arrows) or S4-PDZ^{-}–transduced HUVECs (red). Note the failure to form trailing edges (blue arrows). C, Cell velocity of HUVECs expressing S4 or S4-PDZ^{-} constructs. These values were normalized to the baseline velocity of WT cells. Note decreased velocity of S4-PDZ^{-}–expressing cells (*P=0.0017; S4 PDZ^{-} vs control).

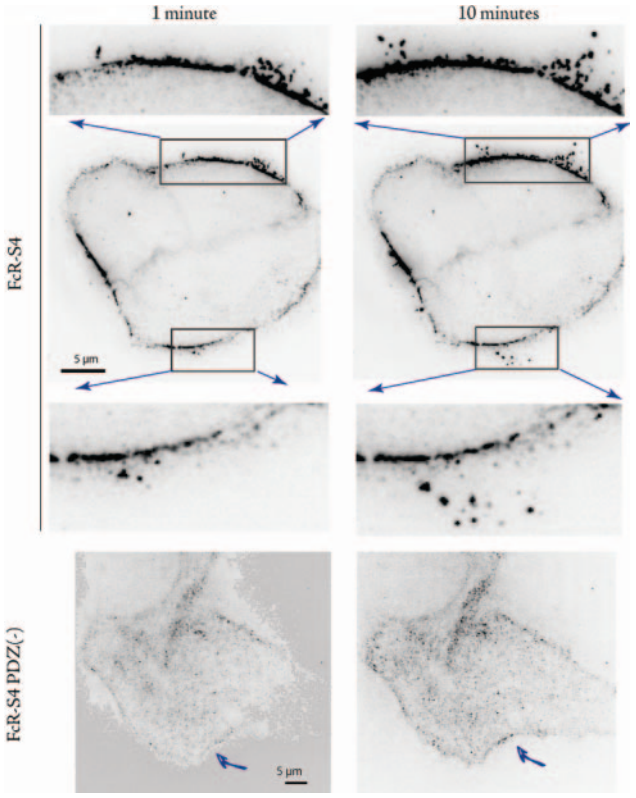

Figure 3. S4 clustering induces PDZ-dependent lamellipodia outgrowth. RFPECs expressing FcR-S4–PDZ$^-$ (left panels) or FcR-S4 (right panels) were F(ab)2 clustered as described. Note the chimera construct redistribution into the leading edge (arrows) of the cell on antibody clustering.

These data suggest that S4 promotes cell migration in response to ligand binding, and that this is specifically dependent on its interaction with synectin.

To monitor endothelial cell migration after binding to fibronectin, a native S4 ligand, full-length S4, and an S4 mutant lacking its PDZ-binding region (S4-PDZ$^-$) constructs were transiently expressed in HUVECs. On attachment to a fibronectin-coated surface, transiently expressed full-length S4 became concentrated in lamellipodia (Figure 2A) in a manner similar to the expression of the endogenous S4 (supplemental Figure II). Interestingly, cells expressing S4-PDZ$^-$, whereas also forming lamellipodia, failed to polarize by form leading and trailing edges and exhibited a significant decrease in random migration velocity when compared with cells expressing GFP or S4 (Figure 2B and 2C).

In unstimulated endothelial cells, S4 is diffusely distributed over the cell surface, whereas ligand clustering leads to the appearance of a punctate distribution.[23,28] To further study the cellular distribution of S4 after antibody clustering, we used TIRF microscopy, a technique that allows detection of a fluorescent signal that originates only from the basal surface of the cell. After antibody clustering, the FcR-S4 chimera localized to cell protrusions on the leading edge (Figure 3, top panels). In contrast, cells expressing the FcR-S4–PDZ$^-$ chimera demonstrated increased basal surface concentrations of the fusion protein subsequent to clustering, without the accompanying formation of cell protrusions (Figure 3, bottom panels). To determine whether defective protrusion in FcR-S4–PDZ$^-$–expressing cells is attributable to a specific disruption of S4 signaling, rather than an artifact of FcR-S4–PDZ$^-$ overexpression, we stimulated cells with EGF. Both FcR-S4 and FcR-S4–PDZ$^-$–expressing endothelial cells responded to EGF by extensive formation of new protrusions (supplemental Figure III).

Among the proteins coordinating the cytoskeletal rearrangements of protrusion formation and subsequent cellular migration is the small GTPase Rac1. We demonstrated previously that antibody clustering of FcR-S4 chimeras leads to Rac1 activation.[23] However, the role of the S4-PDZ–binding domain in this process has not been established. We therefore studied Rac1 activation in FcR-S4– and FcR-S4–PDZ$^-$–expressing RFPECs. Clustering of FcR-S4 cells resulted in significant increase in the amount of GTP-bound Rac1 consistent with Rac1 activation (Figure 4A and 4B). In contrast, FcR-S4–PDZ$^-$ clustering did not significantly increase the amount of GTP-bound Rac1 (Figure 4A and 4B). However, interestingly, RFPECs expressing the PDZ$^-$ mutant construct had much higher levels of activated Rac1 than cells expressing FcR-S4 before clustering. These observations are in agreement with the reported high basal level of activated Rac1 in S4$^{-/-}$ fibroblasts.[14] Thus, S4–PDZ domain protein interaction(s) inhibits Rac1 activation, and clustering of S4 removes this inhibition. An elevated basal level of Rac1 activity in endothelial cells lacking the PDZ-containing protein synectin supports this conclusion (Figure 4C) and suggests the essential role of synectin in S4-mediated regulation of Rac1 activity.

Despite Rac1 activation in FcR-S4–PDZ$^-$–expressing and synectin$^{-/-}$ endothelial cells, they exhibit decreased S4 clustering–mediated cell migration. We hypothesized, therefore, that Rac1 activation in cells expressing S4-PDZ$^-$ mutant does not result in a promigratory phenotype because of incorrect localization of the active form of Rac1. To study the localization of active Rac1 after S4 clustering, two independent monitors of Rac1 activity[32] were transiently overexpressed in FcR-S4 and FcR-S4–PDZ$^-$ cell lines (Figure 5A). One construct enables the direct detection of overexpressed Rac1 activity, whereas the second is used to detect the binding of endogenous Rac1 to the Cdc42 and Rac1 interacting domain. Using both Rac1 activity monitor constructs, we observed polarization of Rac1 activity in FcR-S4–expressing endothelial cells after antibody clustering that preceded formation of trailing and leading edges of the cell (Figure 5B). This led to polarization of 72% of these cells (Figure 5C). In contrast, FcR-S4–PDZ$^-$–expressing cells exhibited diffuse cytoplasmic distribution of activated Rac1 before clustering, consistent with the higher amount of activated Rac1 at baseline in this cells (Figure 5B). After clustering, active Rac1 remained diffusely distributed with only 19% of the cells demonstrating any polarization ($P<0.05$ versus FcR-S4–expressing cells).

Discussion

The principal finding of this study is that S4 clustering on the endothelial cell surface leads to the activation of Rac1 and results in cell polarization and migration in a manner that is dependent on a PDZ-based interaction with a S4 partner

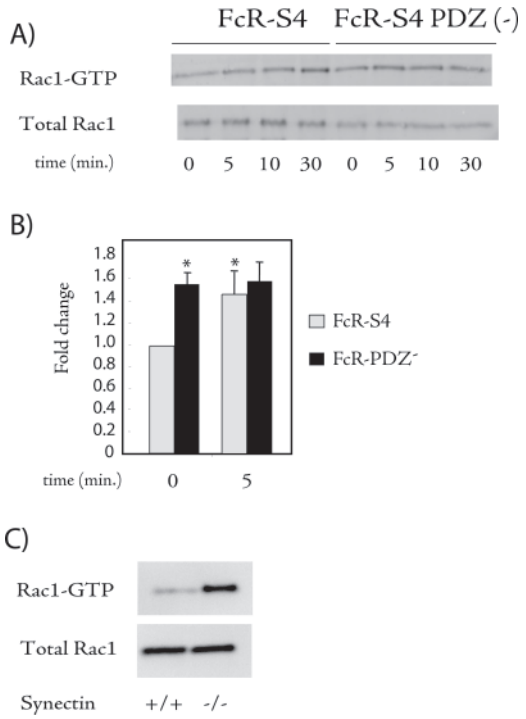

Figure 4. Rac1 activation after S4 clustering is PDZ dependent. Rac1 activity was measured by GST pull-down assays. Total Rac1 expression was used as a control for gel loading. A, Rac1 activity at the indicated time points after clustering of FcR-S4 (left) and FcR-S4–PDZ$^-$ (right) constructs in RFPEC. B, Quantitative analysis of Rac1 GST pull-down assays (n=3). Data are shown mean±SE of the fold change of the unstimulated FcR-S4–expressing RFPECs. *P=0.054 and **P=0.06 (t test). C, Basal Rac1 activity in wild-type and synectin$^{-/-}$ cells. Primary microvascular cells from wild-type and synectin–knockout mice were starved for 24 hours before measuring Rac1 activity, as described above. Note the high basal level of active Rac1 in S4 PDZ$^-$ and synectin$^{-/-}$ cells.

synectin. The expression of the S4 construct lacking a PDZ-binding domain or ablation of synectin expression results in mislocalized high basal level activity of Rac1 and the inability to polarize and initiate migration in response to S4 clustering.

Syndecans have been recognized recently as signaling molecules in their own right,[3] but the extent of their signaling abilities and their molecular mechanisms of interaction are poorly understood. S4 in particular has been reported to be involved in the mediation of cell migration in response to FGF2[7,8] as well as in focal adhesion formation.[2,12] Furthermore, we demonstrated previously that S4 clustering induces Rac1 activation in endothelial cells,[23] whereas its absence has been reported to also increase Rac1 activity.

The present study was performed to reconcile these findings and to explore the functional effect of S4 clustering on endothelial cell migration. Because S4 clustering with its natural ligands such as FGF2 or fibronectin results not only in "pure" S4 signaling but also in signaling events mediated by FGF-R1 and $\alpha5\beta1$ integrin, respectively, we used antibodies against either the extracellular domain of S4 or against the FcR portion of the FcR-S4 chimera. In both cases, S4 clustering induced endothelial cell migration that was comparable in magnitude to that induced by FGF2, a potent stimulator of cell motility.

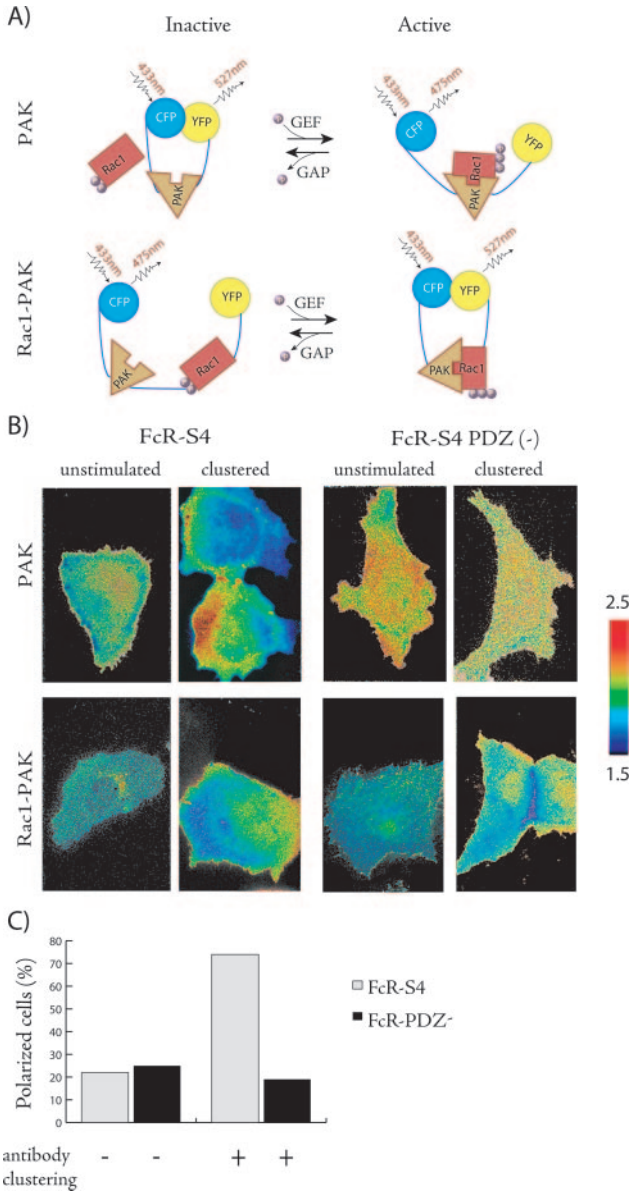

Figure 5. Localization of active Rho GTPases after syndecan clustering. A, Diagram of Rac1 activity monitors. The first monitor (top panels) contains Pak1, flanked by CFP and YFP. This protein construct exhibits an increased intramolecular distance between CFP and YFP on binding to the active form of the listed GTPases, resulting in low-efficiency FRET. The second monitor (bottom panel) contains fused Pak1 and Rac1 with CFP and YFP on its flanks. In this construct, the activation of Rac1 leads to its binding to Pak1, resulting in high-efficiency FRET attributable to the conformation changes of the protein. B, FRET analysis of Rac1 activation in RFPECs expressing FcR-S4 (left panels) and FcR-S4–PDZ$^-$ (right panels). Note a decrease in the FRET signal intensity localizing to the leading edge of the cells expressing PAK (top panel), and an increase in the cells expressed PAK–Rac1 constructs after S4 clustering in FcR-S4. Right panel, Pseudocolor scale of FRET signal. C, Quantitative analysis of cells exhibiting FRET-determined Rac1 polarization. Data are shown as a percentage of cells of FcR-S4 (light gray bars) and FcR-S4–PDZ$^-$ (dark gray bars) before and after antibody S4 clustering. At least 30 cells were analyzed for each experimental condition.

The most prominent features of S4 clustering were rapid cell polarization, including formation of leading and trailing

edges, and S4 concentration on the leading edge and in the lamellipodia within 10 minutes of cell stimulation. This did not occur in cells expressing the S4-PDZ$^-$ chimera, suggesting that S4–PDZ domain–containing protein interaction was required for this event. Furthermore, although in FcR-S4 endothelial cells, S4 clustering led to Rac1 activation and its concentration near the leading edge, in cells expressing the S4-PDZ$^-$ construct, Rac1 remained diffusely distributed. In agreement with these FRET findings, S4 clustering induced migration in FcR-S4– but not FcR-S4–PDZ$^-$–expressing cells. Together, these results demonstrate that S4-PDZ domain–containing protein interaction is required for transport of the activated Rac1 to the leading edge and initiation of cell migration.

The S4-PDZ partner that plays a role in Rac1 activation appears to be the cytoplasmic PDZ-binding protein synectin, originally isolated using a yeast two-hybrid screen with the S4 cytoplasmic domain as a bait.[34] This is a ubiquitously expressed protein[35] involved in regulation of endothelial cell migration and vascular branching morphogenesis.[27] The S4–synectin connection is suggested by the ineffectiveness of S4 clustering to induce migration in synectin$^{-/-}$ endothelial cells. It is also interesting to note that similar to high baseline level of Rac-1 activity in S4-PDZ$^-$ endothelial cells observed in this study, synectin$^{-/-}$ endothelial cells also demonstrate high baseline level of Rac-1 activity.[27] One possible explanation of both S4 clustering–dependent Rac1 activation and the high baseline Rac1 activity in S4-PDZ$^-$ and synectin$^{-/-}$ endothelial cells is that S4 dissociation from synectin on clustering results in removal of associated paxillin, because the S4-paxillin interaction was shown to inhibit Rac1 activation after cell spreading on fibronectin.[36]

Another possibility involves S4 interaction with dynamin 2. Expression of a dominant-negative mutant of dynamin-2 (dynamin-2K44A) affects the trafficking of Rac1, resulting in a high basal level of Rac1 activity attributable to an improper targeting of the active enzyme for recycling.[37] Furthermore, microinjection of dynamin-2K44A impairs epithelial cell ability to form the trailing edge during wound healing.[38] Interestingly, dynamin has been shown to associate with macropinosomal vesicles containing the active form of Rac1,[37] whereas we found that S4 is also present in macropinosomes.[23] Therefore, these similar phenotypes observed in S4-PDZ$^-$–or dynamin-2K44A–expressing cells may be attributable to intracellular targeting of dynamin-2, necessary for migratory cell polarization,[37,38] being dependent on S4–synectin interaction. In this regard, it is interesting to note that a recent study has shown a direct interaction between S4 and dynamin-2.[39]

It is also possible that PKCα plays an important role in the regulation of S4-dependent Rac1 activation. Previous studies have shown that S4 regulates activity and distribution of PKCα,[5] and that this is regulated by phosphorylation state of Ser^{183}.[29,40] Furthermore, the presence of an intact S4-PDZ domain appears necessary for the regulation of Ser^{183} phosphorylation[7] because a PDZ-binding domain mutation results in S4 hyperphosphorylation that inhibits activation of PKCα by potentially preventing S4 multitimerization.[41]

Other proteins, in addition to fibronectin, that have been implicated in promotion of cell spreading in an S4-dependent manner, are disintegrins from the ADAMs family.[42] ADAM12 uses S4 as a primary cell surface receptor to trigger focal adhesions disassembly and β1 integrin–mediated cell spreading. Clustering of another syndecan family member, syndecan-1, also results in the enhanced cell spreading and formation of lamellipodia but is independent of PDZ-binding domain interactions.[43]

In summary, our results suggest that in endothelial cells, S4 forms a complex with a PDZ partner, synectin, that inhibits Rac1 activation. Clustering of S4 locally releases this inhibition, resulting in targeting of the active Rac1 to the leading edge of the cell, thus promoting cell motility. These findings, therefore, establish S4 as a regulator of endothelial cell migration.

Acknowledgments

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