**Background:** Syndecan-1 regulates migration and adhesion in the lung epithelium.

**Results:** The transmembrane domain modulates focal adhesion disassembly, whereas the extracellular domain regulates cell adhesion.

**Conclusion:** Lung epithelial migration is governed by the syndecan-1 transmembrane domain and is independent of the extracellular domain control of cell adhesion.

**Significance:** Syndecan-1 is a receptor that uses multiple domains to facilitate its biological function.

Syndecan-1 is a cell surface proteoglycan that can organize co-receptors into a multimeric complex to transduce intracellular signals. The syndecan-1 core protein has multiple domains that confer distinct cell- and tissue-specific functions. Indeed, the extracellular, transmembrane, and cytoplasmic domains have all been found to regulate specific cellular processes. Our previous work demonstrated that syndecan-1 controls lung epithelial migration and adhesion. Here, we identified the necessary domains of the syndecan-1 core protein that modulate its function in lung epithelial repair. We found that the syndecan-1 transmembrane domain has a regulatory function in controlling focal adhesion disassembly, which in turn controls cell migration speed. In contrast, the extracellular domain facilitates cell adhesion through affinity modulation of $\alpha_\beta_2$ integrin. These findings highlight the fact that syndecan-1 is a multidimensional cell surface receptor that has several regulatory domains to control various biological processes. In particular, the lung epithelium requires the syndecan-1 transmembrane domain to govern cell migration and is independent from its ability to control cell adhesion via the extracellular domain.

The syndecan family of proteoglycans is composed of four mammalian members with unique distribution and function (1, 2). Syndecan-1–4 are type I transmembrane proteins and, like all proteoglycans, are decorated with glycosaminoglycan side chains (3). The distinct tissue and cellular distribution of the various syndecan family members could account for their non-overlapping functions, but increasing data also suggest that the core protein has unique features that tailor specific roles (4–11).

The syndecan proteins are highly conserved across species. However, individual syndecan proteoglycans have distinct features compared with other family members. The ectodomain is the most unique region across the different syndecan proteins, varying in length and sequence homology (1). Although all of the syndecans have heparan sulfate glycosaminoglycans attached to the extracellular domain, syndecan-1 and syndecan-3 also have chondroitin sulfate side chains. The short cytoplasmic tail contains a variable stretch of sequences (V region) flanked by two highly conserved regions (C1 and C2). Finally, the single-pass transmembrane domain, although relatively conserved among family members, has enough differences to confer unique functions (12).

Although found on most cells in the body, syndecan-1 is most abundant on epithelial and plasma cells (13). Shedding of the syndecan-1 ectodomain is an important mechanism for controlling biological processes such as inflammation (14–17). However, syndecan-1 also functions as a cell surface receptor and governs in vivo processes such as wound repair, gut barrier function, and lipid metabolism (18–23). In contrast to homeostatic functions, malignant cells also co-opt syndecan-1 to regulate cell invasion, angiogenesis, and tumorigenesis (8, 10, 11, 24).

Syndecan-1 has cell- and tissue-specific functions. A prime example is the fact that syndecan-1 augments epithelial cell migration in skin and cornea but restrains migration in lungs (18–20, 25). The divergent actions of syndecan-1 are thought to be largely controlled by the cellular context, where the appropriate co-receptor is presented along with syndecan-1 to create a signaling complex that can then transduce extracellular clues to modulate cellular function (26). Accordingly, the multiple domains of syndecan-1 have different effects in regulating these processes. Although some studies have demonstrated that the extracellular domain facilitates cell spreading (4, 9, 10), others
have found that the syndecan-1 cytoplasmic domain is the requisite portion (27–29).

Our previous work showed that syndecan-1 regulates both cell adhesion and migration (18). Here, we used wild-type and mutant syndecan-1 constructs to map out the relevant domains of the core protein that govern the phenotypic response in the lung epithelium. We demonstrate that the syndecan-1 extracellular domain controls cell adhesion through \( \alpha_2 \beta_1 \) integrin affinity modulation. However, the extracellular domain by itself has no effect on cell migration and requires the transmembrane domain to control migration speed and focal adhesion disassembly.

**EXPERIMENTAL PROCEDURES**

**Cloning**—The creation of mutant syndecan-1 was described previously (8, 11, 30). cDNAs of various mouse syndecan-1 constructs were subcloned into adeno-associated virus (AAV) \(^2\)-internal ribosome entry site (IRES)-enhanced GFP (eGFP), AAV-IRES-mCherry, and pBMN-IRES-blasticidin vectors using BamHI and XhoI digestion. AAV vectors with the respective mutant mouse syndecan-1 were produced following the manufacturer’s protocol (AAV-DJ helper-free expression system, Cell Biolabs, San Diego, CA). Retroviral vectors were created as described previously using the PhiX packaging cell line (18). All subcloned plasmid DNA sequences were confirmed by DNA sequencing.

**Cell Culture**—BEAS-2b cells, a non-malignant immortalized human bronchial epithelial cell line, were cultured in fully supplemented bronchial epithelial growth medium (Lonza, Walkersville, MD). The creation of BEAS-2b cells stably expressing human syndecan-1 shRNA (B2bshRNA.hSdc1) or scrambled control shRNA (B2bshRNA.scr) was validated previously (18). AAV transduction was used for transient overexpression of mutant mouse syndecan-1. Retroviral vectors were used to stably transduce mutant mouse syndecan-1, and cells were maintained in bronchial epithelial growth medium plus blasticidin (10 \( \mu \)g/ml).

**Migration Assay**—Monolayers of B2bshRNA.scr and B2bshRNA.hSdc1 cells were plated on No. 1.5 chambered cover-glass (Corning, Union City, CA) coated with rat tail type I collagen (2 \( \mu \)g/cm\(^2\); BD Biosciences). Monolayers were wounded with a sterile P100 pipette tip, and migration was observed under a Nikon TiE inverted widefield fluorescence microscope, which has a humidified chamber to maintain cells at 37 °C and 5% CO\(_2\). Differential interference contrast images were obtained every 10–20 min for up to 12 h using a CFI 60×/1.49 NA Apo TIRF oil immersion objective. Migration speed was determined by manually measuring the distance traveled by the cell front over time using Nikon Elements AR software. In transient transduction experiments, cells expressing mutant syndecan-1 were identified by coexpression of eGFP.

**Focal Adhesion Disassembly Assays**—B2bshRNA.scr and B2bshRNA.hSdc1 cells stably expressing paxillin-eGFP were created previously (31). Cells were transduced with an AAV vector to coexpress mouse syndecan-1 and mCherry. To determine the focal adhesion disassembly rate in migrating cells, monolayers of cells were injured and observed by total internal reflection fluorescence (TIRF) microscopy using a CFI 60×/1.49 NA Apo TIRF oil immersion objective. Additionally, focal adhesion disassembly was induced with a nocodazole washout assay (32, 33). The fluorescence intensity of the focal adhesion was measured over the entire life span (NIH ImageJ). All data were normalized so that the fluorescence intensity was a percentage of the maximum intensity for the individual focal adhesion. The rate of focal adhesion disassembly was determined for mCherry-expressing cells as described previously (31, 32, 34).

**Fluorescence Recovery after Photobleaching**—A Nikon A1R scanning laser confocal microscope using a CFI 60×/1.40 NA Plan Apo VC oil immersion objective fitted with a Tokai Hit stage-top

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2 The abbreviations used are: AAV, adeno-associated virus; IRES, internal ribosomal entry site; eGFP, enhanced GFP.
incubator to maintain cells in a humidified chamber at 37 °C and 5% CO₂ was used for fluorescence recovery after photobleaching experiments. Cells were transduced with an AAV vector to coexpress mouse syndecan-1 and mCherry. Migrating B2b<sub>shRNA.scr</sub> and B2b<sub>shRNA.hSdc1</sub> cells stably expressing paxillin-eGFP had leading edge focal adhesions selectively photobleached in mCherry-expressing cells. With the 405 and 488 nm lasers set to 50% power, a 1-s pulse was applied over the entire focal adhesion, which would consistently photobleach the focal adhesion to 80% of the prebleaching intensity. The fluorescence recovery was measured with Nikon Elements AR software, and the half-time of recovery was determined as described previously (31).

FIGURE 2. Transmembrane domain of syndecan-1 regulates migration speed. A, schematic of wild-type and mutant syndecan-1 used in these experiments. B, B2b<sub>shRNA.scr</sub> and B2b<sub>shRNA.hSdc1</sub> cells transduced as indicated were injured, and migration of eGFP cells was observed. White asterisks identify the same cell at 0 and 8 h after injury. See supplemental Video 2 for the entire image series. Scale bar = 20 μm.
Syndecan-1 Domains in Migration and Adhesion

**TABLE 1**

| Syndecan-1 domains and migration speed |
|---------------------------------------|
| B2bshRNA.scr and B2bshRNA.hSdc1 + mSdc1-GPI cells were significantly faster than B2bshRNA.scr cells by one-way ANOVA and Bonferroni post hoc analysis (p < 0.01; n = three independent experiments). Migration speed for a minimum of 10 cells was measured for each condition in each experiment. All data are presented as means ± S.E. |

| Cell line | Migration speed μm/h |
|-----------|----------------------|
| B2bshRNA.scr | 4.57 ± 0.40 |
| B2bshRNA.hSdc1 | 6.45 ± 0.48 |
| B2bshRNA.hSdc1 + mSdc1 | 3.67 ± 0.36 |
| B2bshRNA.hSdc1 + mSdc1-CT1 | 4.07 ± 0.34 |
| B2bshRNA.hSdc1 + mSdc1-GPI | 6.47 ± 0.44 |

**RESULTS**

Syndecan-1 Transmembrane Domain Restrains Cell Migration—Syndecan-1 restrains migration of lung epithelial cells (18). Here, we used shRNA to suppress syndecan-1 expression in lung epithelial cells and found that cells with suppressed syndecan-1 expression (B2bshRNA.hSdc1) had a faster migration speed than cells expressing a control shRNA (B2bshRNA.scr) (7.56 ± 0.47 μm/h versus 4.99 ± 0.44 μm/hr; p < 0.0005) (Fig. 1 and supplemental Video 1).

To map the domains of the syndecan-1 core protein that control cell migration, we expressed wild-type and mutant syndecan-1 cDNAs into B2bshRNA.hSdc1 cells and compared their migration speed with that of control B2bshRNA.scr and B2bshRNA.hSdc1 cells (eGFP expression only). In contrast to full-length syndecan-1 (mSdc1, 311-amino acid core protein), mSdc1-CT1 lacked the cytoplasmic domain (amino acids 1–279), and mSdc1-GPI lacked both the transmembrane and cytoplasmic domains (amino acids 1–252) (Fig. 2A) (10). The entire syndecan-1 extracellular domain was present in all of the constructs. The expression levels of full-length and mutant mSdc1 proteins were similar under all conditions (supplemental Fig. S1).

We found that B2bshRNA.hSdc1 cells migrated faster than B2bshRNA.scr cells (Fig. 2B, Table 1, and supplemental Video 2). Both mSdc1 and mSdc1-CT1 slowed migration of B2bshRNA.hSdc1 cells to a similar rate compared with B2bshRNA.scr cells. Only mSdc1-GPI, which contains the entire syndecan-1 ectodomain but is anchored to the cell membrane by a glycosylphosphatidylinositol domain (11, 35–37), lacked the ability to slow cell migration (Table 1), indicating that the transmembrane region is the crucial domain regulating lung epithelial migration.

To ensure that the glycosylphosphatidylinositol anchor did not have any inhibitor actions on syndecan-1, we used a mutant syndecan-1 that had polyleucine substitutions in the transmembrane domain (mSdc1-pL) (supplemental Fig. S2) (38). In concordance with the data from the mSdc1-CT1 and mSdc1-GPI cells were significantly different from B2bshRNA.scr cells by one-way ANOVA and Bonferroni post hoc analysis (p < 0.001; n = four independent experiments). The slope of the descending limb of the normalized intensity curve (Figs. 3A and 3F) reflects the disassembly rate. A more negative value equates faster focal adhesion disassembly. A minimum of 10 focal adhesions were measured for each condition in each experiment. mSdc1-GPI cells were significantly different from B2bshRNA.scr cells by one-way ANOVA and Bonferroni post hoc analysis (p < 0.01; n = three independent experiments).

**SYNOPSIS**

Syndecan-1 is a transmembrane glycosylphosphatidylinositol-anchored proteoglycan that acts as a coreceptor for a variety of ligands, including fibroblast growth factor-2 (FGF-2). While it has been shown that syndecan-1 expression is required for lung epithelial cell migration, the mechanism by which it regulates migration is not well understood. Here, we find that syndecan-1 expression attenuates focal adhesion turnover and slows cell migration. Our findings suggest that syndecan-1 regulates cell migration in part by inhibiting arrestin recruitment and focal adhesion turnover.

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GPI mutants, the mSdc1-pL mutant did not slow migration of B2b
shRNA.hSdc1 cells. Syndecan-1 Transmembrane Domain Controls Focal Adhesion Disassembly—Cell migration speed is largely determined by the focal adhesion turnover rate (32, 37, 39). Our previous work demonstrated syndecan-1 restrains cell migration by slowing focal adhesion disassembly (31). Therefore, we evaluated the disassembly of focal adhesions in migrating cells (Fig. 3). Kymographs of individual focal adhesions best represented the turnover through time (Fig. 3A). Moreover, the intensity of the focal adhesion was measured, and the downward slope of intensity correlated with the disassembly rate (Fig. 3B) (31, 32, 37). Migrating B2b
shRNA.hSdc1 cells were compared with B2b
shRNA.scr cells and had a significantly shorter focal adhesion life span and faster disassembly (Table 2), consistent with our prior observations (31). Whereas mSdc1-GPI had no effect, mSdc1 and mSdc1-CT1 both reversed the focal adhesion life span and disassembly differences in B2b
shRNA.hSdc1 cells to become more similar to those in B2b
shRNA.scr cells, which trends with the pattern seen with the migration speed.

Microtubules target focal adhesions and induce disassembly (32, 33). Forced disassembly of focal adhesions occurred within minutes of microtubule polymerization after nocodazole washout (Fig. 4, A–E). The intensity of the focal adhesion was measured (Fig. 4F), and the disassembly rate was calculated (Table 2). B2b
shRNA.hSdc1 cells had faster disassembly of focal adhesions compared with B2b
shRNA.scr cells. These findings are congruous with the focal adhesion disassembly rate of migrating cells (31). Moreover, the transmembrane domain once again was the relevant domain that could reverse the accelerated focal adhesion disassembly rate in B2b
shRNA.hSdc1 cells (Table 2).

Syndecan-1 Transmembrane Domain Facilitates Exchange of Adhesion Complex Proteins—Cycling of adhesion complex proteins (e.g. paxillin and focal adhesion kinase) between focal adhesions and the cytosol controls migration speed by altering the stability of the focal adhesions (31–34, 40). Fluorescence recovery after photobleaching was used to evaluate the exchange rate of adhesion complex proteins (Fig. 5, A and B). Consistent with our previous observations (31), the dynamic cycling of paxillin was faster in B2b
shRNA.scr cells than in B2b
shRNA.hSdc1 cells (Table 3). The kinetics of adhesion complex protein exchange was reversed and similar to those in B2b
shRNA.scr cells only when mSdc1 and mSdc1-CT1 were introduced into B2b
shRNA.hSdc1 cells. Syndecan-1 Ectodomain Is Required for Integrin Activation—Changes to the integrin affinity state can modulate cell migration (32, 41). Because syndecan-1 regulates both cell migration and α5β1 integrin activation (18), we evaluated the relevant domains of syndecan-1 that control α5β1 integrin activation. Using activation state-specific antibodies, we quantified the level of the high affinity β1 integrin subunit (Fig. 6). The high

FIGURE 4. Syndecan-1 transmembrane domain attenuates focal adhesion disassembly after nocodazole washout. Cells stably expressing paxillin-eGFP were treated with nocodazole (10 μM, 4 h), and the disassembly of focal adhesions was evaluated after washout. A–E, focal adhesion disassembly was observed in B2b
shRNA.scr cells, B2b
shRNA.hSdc1 cells, and B2b
shRNA.hSdc1 cells expressing wild-type and mutant mouse syndecan-1. F, intensity curves of the focal adhesions in A–E.
affinity β1 subunit was less abundant in B2bshRNA.hSdc1 cells compared with B2bshRNA.scr cells, replicating our prior findings (18). However, in contrast to cell migration, where the transmembrane domain regulated migration speed and focal adhesion dynamics, integrin activation was controlled by the syndecan-1 ectodomain. Indeed, mSdc1, mSdc1-CT1, and mSdc1-GPI were all capable of increasing the level of the activated β1 integrin subunit in B2bshRNA.hSdc1 cells compared with B2bshRNA.scr cells (Fig. 6).

Functionally, we determined whether the syndecan-1 ectodomain also controls cell adhesion to type I collagen. As expected, B2bshRNA.scr cells were less adherent than B2bshRNA.scr cells (Table 4) (9, 18). However, adhesion of B2bshRNA.scr cells was augmented with the expression of mSdc1, mSdc1-CT1, and mSdc1-GPI, all of which contain the entire syndecan-1 ectodomain.

**DISCUSSION**

After epithelial injury, cell-cell and cell-matrix interactions form a contextual framework vital to guiding and optimizing collective cell migration (42, 43). Although the cells in an epithelial surface migrate in concert while maintaining their cellular interconnections, each individual cell retains characteristics of single-cell migration (44). Syndecan-1 restrains cell migration and facilitates the activation of αβ1 integrin (18). Here, we have demonstrated that syndecan-1 regulates cell migration independent of the ability to control the αβ1 integrin activation state. The transmembrane domain is the critical portion of the syndecan-1 core protein that controls cell migration by controlling focal adhesion disassembly. In contrast, cell adhesion is
Syndecan-1 is a multidimensional protein that coordinates several events for a specific cellular function. For example, α2β1 integrin and insulin-like growth factor receptor-1 coalesce through syndecan-1 to form a fully functional ternary complex (47). Furthermore, murine B82L fibroblasts send out filopodial projections upon syndecan-1 ligation but also require α5β1 integrin engagement for a spread phenotype (7). Both the extracellular and transmembrane domains are necessary to induce cell spreading and polarization in Raji lymphoid cells (38). Additionally, the syndecan-1 extracellular domain can augment cell adhesion but in itself is not sufficient to promote cell spreading and invasion (9, 10).

Our data show that syndecan-1 truncated at the first cytoplasmic amino acid but retaining the endogenous transmembrane domain (i.e. mSdc1-CT1) is functionally conserved. In contrast, substitutions with a glycosylphosphatidylinositol anchor or with polyleucines in the transmembrane domain both lose the ability to control cell migration. Because the transmembrane domain is believed to facilitate dimerization of the syndecan receptor (1), syndecan-1 may require the transmembrane domain to dimerize in mediating its effects on cell migration. Of particular note, glycine-to-leucine mutations within the transmembrane domain of syndecan-2 and syndecan-4 abrogate both dimerization and effects on migration (48).

Syndecan-1 Domains in Migration and Adhesion

FIGURE 6. Syndecan-1 ectodomain regulates activation of β, integrin subunit. Shown are representative histograms of the high affinity β, integrin subunit using antibody clones TS2/16 (A) and 12G10 (B). The cell line and geometric mean fluorescence intensity (MFI) are indicated to the right of the respective histogram.

TABLE 4
Syndecan-1 domains and cell adhesion

| Cell line                  | Normalized adhesion |
|----------------------------|---------------------|
| B2bshRNA.scr               | 99.81 ± 2.30        |
| B2bshRNA.sSdc1             | 63.42 ± 6.02        |
| B2bshRNA.sSdc1 + mSdc1     | 90.42 ± 11.04       |
| B2bshRNA.sSdc1 + mSdc1-CT1 | 88.93 ± 7.00        |
| B2bshRNA.sSdc1 + mSdc1-GPI | 87.82 ± 8.02        |

* B2bshRNA.sSdc1 cells were significantly less adherent than other cells by one-way ANOVA (p < 0.005; n = four independent experiments). The percentage of adherent cells under all conditions was normalized to the percentage of adherent B2bshRNA.scr cells.

Controlled by the syndecan-1 ectodomain through integrin affinity modulation.

Various functions have been identified for the syndecan-1 extracellular domain. Angiogenesis and cancer cell invasion are regulated by the syndecan-1 ectodomain (8, 10, 11, 45). Additionally, the syndecan-1 extracellular domain is required for cell adhesion and spreading (4, 8–10). Affinity modulation of the α2β1 and α5β1 integrins also requires the extracellular domain (5, 7, 8). Moreover, the heparan sulfate side chains are required to engage the matrix, but distinct portions of the syndecan-1 core protein are required for its cell-specific functions (5, 27, 28). Although heparan sulfate can bind collagen, this interaction is most likely not the main determinant of cell adhesion (9, 10, 46). These findings are consistent with ours, which indicate that the syndecan-1 extracellular domain mediates the adhesive properties of the cells. Furthermore, at least in the lung epithelium, syndecan-1 facilitates cell adhesion through affinity modulation of α2β1 integrin (18).

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Our data show that syndecan-1 truncated at the first cytoplasmic amino acid but retaining the endogenous transmembrane domain (i.e. mSdc1-CT1) is functionally conserved. In contrast, substitutions with a glycosylphosphatidylinositol anchor or with polyleucines in the transmembrane domain both lose the ability to control cell migration. Because the transmembrane domain is believed to facilitate dimerization of the syndecan receptor (1), syndecan-1 may require the transmembrane domain to dimerize in mediating its effects on cell migration. Of particular note, glycine-to-leucine mutations within the transmembrane domain of syndecan-2 and syndecan-4 abrogate both dimerization and effects on migration (48).
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Unlike syndecan-2, -3, and -4, which form strong homo- and heterodimers, the syndecan-1 transmembrane domain has relatively weak interactions with itself and other members of the syndecan family (12). Thus, alternatively, the transmembrane domain may not necessarily mediate syndecan-1 function through dimerization but through organizing into distinct cell surface domains. Indeed, Raji cells require lipid rafts for syndecan-1-mediated spreading (38). Syndecan-1 may coalesce in specific membrane pools, bringing it in proximity to other co-receptors to form a multimeric complex required to carry out specific membrane pools, bringing it in proximity to other co-receptors to form a signaling complex that transduces signals to augment cell adhesion and restrain cell migration. Although these results further substantiate the notion that cells use multiple syndecan-1 domains to fully transduce signals from the extracellular matrix into the cell.

Cell migration speed can be controlled by altering the focal adhesion turnover rate (39). Cells can also alter the optimum substrate concentration by regulating focal adhesion dynamics (32, 37). Syndecan-1 restrains cell migration by attenuating focal adhesion disassembly, which prolongs focal adhesion life span and slows migration speed (31). Here, we have shown that the syndecan-1 transmembrane domain regulates cell migration. Moreover, our fluorescence recovery after photobleaching experiments demonstrate that the transmembrane domain controls the cycling of adhesion complex proteins, which regulates focal adhesion turnover. Our findings are consistent with the fact that faster exchange of focal adhesion proteins slows migration by attenuating focal adhesion turnover (31, 34, 40, 54, 55).

In sum, syndecan-1 most likely interacts with one or more co-receptors to form a signaling complex that transduces signals to augment cell adhesion and restrain cell migration. Although the extracellular domain is necessary for integrin affinity modulation, the transmembrane domain is crucial for controlling focal adhesion disassembly and migration speed in wounded lung epithelial cells. These data support the notion that syndecan-1 coordinates co-receptor complex formation through various domains of its core protein to mediate cellular function. The specific functional outcome is largely determined by the cellular context and regulated coexpression of signaling receptors.

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