Role of c-Abl tyrosine kinase in smooth muscle cell migration

Rachel A. Cleary, Ruping Wang, Omar Waqar, Harold A. Singer, and Dale D. Tang

Center for Cardiovascular Sciences, Albany Medical College, Albany, New York

Submitted 21 October 2013; accepted in final form 17 January 2014

Cleary RA, Wang R, Waqar O, Singer HA, Tang DD. Role of c-Abl tyrosine kinase in smooth muscle cell migration. Am J Physiol Cell Physiol 306: C753–C761, 2014. First published January 29, 2014; doi:10.1152/ajpcell.00327.2013.—c-Abl is a nonreceptor protein tyrosine kinase that has a role in regulating smooth muscle cell proliferation and contraction. The role of c-Abl in smooth muscle cell migration has not been investigated. In the present study, c-Abl was found in the leading edge of smooth muscle cells. Knockdown of c-Abl by RNA interference attenuated smooth muscle cell motility as evidenced by time-lapse microscopy. Furthermore, the actin-associated proteins cortactin and profilin-1 (Pfn-1) have been implicated in cell migration. In this study, cell adhesion induced cortactin phosphorylation at Tyr-421, an indication of cortactin activation. Phosphorylated cortactin and Pfn-1 were also found in the cell edge. Pfn-1 directly interacted with cortactin in vitro. Silencing of c-Abl attenuated adhesion-induced cortactin phosphorylation and Pfn-1 localization in the cell edge. To assess the role of cortactin/Pfn-1 coupling, we developed a cell-permeable peptide. Treatment with the peptide inhibited the interaction of cortactin with Pfn-1 without affecting cortactin phosphorylation. Moreover, treatment with the peptide impaired the recruitment of Pfn-1 to the leading edge and cell migration. Finally, β1-integrin was required for the recruitment of c-Abl to the cell edge. Inhibition of actin dynamics impaired the spatial distribution of c-Abl. These results suggest that β1-integrin may recruit c-Abl to the leading cell edge, which may regulate cortactin phosphorylation in response to cell adhesion. Phosphorylated cortactin may facilitate the recruitment of Pfn-1 to the cell edge, which promotes localized actin polymerization, leading edge formation, and cell movement. Conversely, actin dynamics may strengthen the recruitment of c-Abl to the leading edge.

SMOOTH MUSCLE CELL MIGRATION plays an essential role in regulating development and homeostasis of the respiratory and cardiovascular systems and contributes to the development of many pathological processes such as airway/vascular remodeling (13–15). During migration, cells form leading edge in response to guidance cues and adhesive proteins in the extracellular matrix. Formation of the leading cell edge is driven by local actin filament assembly (27). However, the mechanisms that regulate localized actin dynamics are not completely elucidated.

c-Abl (Abelson tyrosine kinase, Abl) is a nonreceptor tyrosine kinase that has a role in the regulation of the actin cytoskeleton important for various cellular functions including cell adhesion, proliferation, growth, and development (16, 19, 28, 36). In smooth muscle, c-Abl is necessary for force development in response to contractile activation (2, 8, 18). However, the role of c-Abl in nonmuscle cell migration is controversial. c-Abl has been shown to promote motility of myeloid cells and cancer cells (4, 17, 21). In contrast, c-Abl kinase was reported to inhibit the motility of embryonic fibroblasts (20). The functional role of c-Abl in smooth muscle cell migration has not been investigated.

Cortactin is an adapter protein that has a role in regulating actin dynamics and cell motility. Cortactin undergoes phosphorylation at Tyr-421 in response to external stimulation, which has been implicated in its activation (1, 9). Cortactin may regulate actin polymerization by controlling the activation of N-WASP (neuronal Wiskott-Aldrich syndrome protein), an actin polymerization promoter (1, 9). In addition, profilin-1 (Pfn-1) is an actin-regulatory protein that is capable of modulating actin dynamics, cell migration, and smooth muscle contraction (11, 30, 32, 33). Pfn-1 promotes actin polymerization by catalyzing the exchange of actin-bound ADP for ATP and by releasing actin monomer from thymosin-B4; both processes facilitate unidirectional addition of G-actin to F-actin (12, 29, 30). Although these proteins have been implicated in cell migration, the interactions of c-Abl with cortactin and Pfn-1 in the cellular process have not been investigated.

β1-Integrin is highly expressed in smooth muscle cells and couples with α-subunits to form transmembrane receptors that link the actin cytoskeleton to extracellular matrix. In addition to mechanical transmission and cell signaling, β1-integrin has also been implicated in cell migration (25, 29, 30).

Actin polymerization may mediate intracellular trafficking of the glucose transporter GLUT4. In adipocytes and striated muscle cells, GLUT4 undergoes spatial translocation to the plasma membrane from the cytoplasm upon insulin activation, which may promote glucose uptake. Inhibition of actin polymerization by molecular approaches attenuates the intracellular trafficking of GLUT4 during insulin activation (3). The potential role of actin dynamics in c-Abl spatial localization has not been explored.

The objective of this study was to evaluate the role of c-Abl in smooth muscle cell migration during adhesion to matrix proteins, a known extracellular cue to induce cell migration. Furthermore, we evaluated the interactions of c-Abl with cortactin and Pfn-1 in the cellular process. Finally, we assessed whether β1-integrin and actin polymerization have a role in controlling the spatial localization of c-Abl during cell adhesion.

MATERIALS AND METHODS

Cell culture. Human airway smooth muscle (HASM) cells were prepared from human airway smooth muscle tissues that were obtained from the International Institute for Advanced Medicine. Human tissues were nontransplantable and consented for research. This study was approved by the Albany Medical College Committee on Research Involving Human Subjects. Briefly, muscle tissues were incubated for 20 min with dissociation solution [130 mM NaCl, 5 mM KCl, 1.0 mM CaCl2, 1.0 mM MgCl2, 10 mM HEPES, 0.25 mM EDTA, 10 mM d-glucose, 10 mM tauro, pH 7.4, 5 mg collagenase (type I), 10 mg papain (type IV), 1 mg/ml BSA, and 1 mM dithiothreitol (DTT)]. All enzymes were obtained from Sigma-Aldrich. The tissues were then
washed with HEPES-buffered saline solution (composition in mM: 10 HEPES, 130 NaCl, 5 KCl, 10 glucose, 1 CaCl$_2$, 1 MgCl$_2$, 0.25 EDTA, and 10 taurine, pH 7). The cell suspension was mixed with Ham's F12 medium supplemented with 10% (vol/vol) fetal bovine serum (FBS) and antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin). Cells were cultured at 37°C in the presence of 5% CO$_2$ in the same medium. The medium was changed every 3–4 days until the cells reached confluence, and confluent cells were passaged with trypsin/EDTA solution (19, 23, 24, 36).

**Immunoblot analysis.** Cells were lysed in SDS sample buffer composed of 1.5% DTT, 2% SDS, 80 mM Tris-HCl (pH 6.8), 10% glycerol, and 0.01% bromophenol blue. The lysates were boiled in the buffer for 5 min and separated by SDS-PAGE. Proteins were transferred to a nitrocellulose membrane. The membrane was blocked with bovine serum albumin or milk for 1 h and probed with use of primary antibody followed by horseradish peroxidase-conjugated secondary antibody (Fisher Scientific). Proteins were visualized by enhanced chemiluminescence (Fisher Scientific) using the LAS-4000 Fuji Image System. Antibodies against c-Abl, phospho-cortactin (Tyr-421), cortactin, and β$_1$-integrin were purchased from Pierce Biotechnology and Cell Signaling. Pfn-1 antibody was purchased from Sigma-Aldrich and Santa Cruz Biotechnology. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody was purchased from Fitzgerald (Acton, MA). The levels of total protein or phosphoprotein were quantified by scanning densitometry of immunoblots (Fuji Multigauge Software). The luminescent signals from all immunoblots were within the linear range.

**Lentivirus-mediated RNAi and gene transduction in cells.** HASM cells expressing c-Abl short hairpin RNA (shRNA) or control shRNA were generated as previously described (19, 36).

**Cell transfection.** To knockdown β$_1$-integrin expression, cells were transfected with 1 μM β$_1$-integrin sense or antisense oligonucleotides using the Fugene HD transfection reagent kit (Promega) and analyzed 2 days after transfection. The sequence of β$_1$-integrin sense was 5'-GGCCGGGGCAGTCTGATGAGTG-3', whereas the sequence of β$_1$-integrin antisense was 5'-CACTCAGAGTCCCAGCCG-3'.

**Immunofluorescent and fluorescent analysis.** Cells were plated on dishes containing collagen-coated coverslips for 30 min. Immunofluorescent microscopy was used to evaluate the high-resolution digital fluorescent microscope (Leica DMI6000). The times of image capturing, intensity gaining, and image black levels were optimally adjusted and kept constant for all experiments to standardize the fluorescence intensity measurements among experiments.

**Time-lapse microscopy.** Cell motility was evaluated by time-lapse microscopy. Cells were plated in six-well culture dishes with Ham’s F12 medium supplemented with 10% FBS until they reached 20–30% of confluence. Culture dishes were then placed in a chamber at 37°C and filled with 5% CO$_2$. Cell migration was recorded live every 10 min for 16 h with the use of a Leica DMI6000 microscope system. A ×10/0.3 phase-contrast objective was used for image capture. The NIH ImageJ software was used to quantitatively assess net distance, total distance, velocity, and directionality.

**Far Western analysis.** Far Western analysis was performed as previously described (35, 36). Extracts of human lung tissues or cells were immunoprecipitated with cortactin antibody. Cortactin immunoprecipitates were separated by SDS-PAGE followed by membrane transfer. The membranes were incubated with purified Pfn-1 for 2 h at
room temperature and then probed with the use of cortactin antibody and Pfn-1 antibody.

In vitro kinase assay. Purified cortactin was resuspended in kinase buffer containing 10 mM HEPES, pH 7.4, 3 mM MnCl2, and 2 mM DTT, and then purified c-Abl (100 ng; Life Technologies) was added. Kinase reaction was initiated by the addition of 100 μM ATP at 30°C (with shaking) for 30 min and stopped by the addition of SDS sample buffer. The reaction mix was separated by SDS-PAGE followed by membrane transfer. The membrane was probed with phospho-cortactin antibody (Tyr-421), stripped, and reprobed with cortactin antibody.

Preparation of a cell-permeable decoy peptide. A cell-permeable decoy peptide (CTTN-I) was designed to disrupt the interaction of cortactin with Pfn-1. The sequence of CTTN-I peptide is near the NH2 terminus of the proline-rich domain of cortactin (sequence is TEER-LPSSPVYEDAASFKA; NCBI accession no. NM_005231). This peptide may compete with endogenous cortactin for Pfn-1 binding (30, 38). The NH2-terminal end of the peptide was fused with TAT sequence (GRKRRQRRRPPQ) for cell permeability. TAT peptide is a short polybasic sequence derived from the human immunodeficiency virus TAT protein; it has been shown to successfully deliver a large variety of cargos (from small particles to peptides and proteins) into intact cells/tissues (7). The peptide was synthesized by Invitrogen. A peptide with scramble sequence (EPSETSVEYDEARAL-PSSPVYEDAASFKAE; NCBI accession no. NM_005231). This peptide may compete with endogenous cortactin for Pfn-1 binding.

Statistical analysis. All statistical analysis was performed using Prism 6 software (GraphPad Software, San Diego, CA). Comparison among multiple groups was performed by one-way analysis of variance followed by Tukey’s multiple comparison test. Differences between pairs of groups were analyzed by Student-Newman-Keuls test or Dunn’s method. Values of α refer to the number of experiments used to obtain each value. P < 0.05 was considered to be significant.

RESULTS

c-Abl is localized in the leading edge of smooth muscle cells. During the early stage of migration, cells form the leading edge, which is essential for directed cell movement. c-Abl is a nonreceptor protein tyrosine kinase that has a role in smooth muscle contraction and cell proliferation (2, 18, 19, 36). As described earlier, the role of c-Abl in nonmuscle cell migration is controversial. We hypothesized that c-Abl may be localized in the leading edge, which may promote leading edge formation and smooth muscle cell migration. To test this, HASM cells were plated on collagen-coated coverslips for 30 min, and the spatial localization of c-Abl was evaluated by immunofluorescent microscopy. c-Abl was found in the leading edge of smooth muscle cells (Fig. 1A).

c-Abl is pivotal for smooth muscle cell migration. To evaluate whether c-Abl has a role in smooth muscle cell motility, we generated stable c-Abl knockdown (KD) cells and cells

---

Fig. 2. Cortactin undergoes phosphorylation upon cell adhesion and interacts with profilin-1 (Pfn-1) in vitro and in cells. A: adhesion to extracellular matrix promotes cortactin phosphorylation at Tyr-421 in cells. Cells were plated on collagen-uncoated or coated dishes for 30 min. Cortactin phosphorylation in these cells was evaluated by immunoblotting using phospho-cortactin (p-Cort; Tyr-421) antibody and total cortactin antibody. Cortactin phosphorylation in cells treated with coated surfaces is normalized to that in uncoated surfaces. Values represent means ± SE (n = 6). *P < 0.05. B: far Western analysis of cortactin interaction with Pfn-1 in vitro. Cortactin immunoprecipitates were separated by SDS-PAGE and transferred to membranes. Lane a, immobilization of cortactin (Cort) on the membrane probed using cortactin antibody. Lane b, detection of Pfn-1 on immobilized cortactin suggests the association of cortactin with Pfn-1 in vitro. Lane c, no detection of Pfn-1 in the control membrane. Lane d, minor detection of Pfn-1 on the immobilized Y421F cortactin. Immunoblots (IB) are representative of 4 identical experiments. C: colocalization of c-Abl, cortactin, and Pfn-1 in the leading edge. The spatial distribution of these molecules in adherent cells was evaluated by immunofluorescent microscopy. Arrows indicate leading edge. Scale bar, 10 μm. p-Cort, phosphorylated cortactin. D: cells were plated on collagen-uncoated (a) or coated (b) coverslips for 30 min. Antibodies against phosphorylated cortactin was used to assess morphology of the cells. Images are representatives of 35 cells. Arrows indicate leading edge. Scale bar, 10 μm.
expressing scramble (control) shRNA using lentivirus-mediated gene transduction as previously described (19, 36). Immunoblot analysis verified c-Abl KD in the cells (Fig. 1B). Migration of these cells was evaluated by time-lapse microscopy. Uninfected cells and cells expressing control shRNA moved quicker and farther than c-Abl KD cells. Quantification analysis showed that the net and total paths of c-Abl KD cell movement were shorter compared with those of uninfected...
cells and cells expressing control shRNA. Consequently, directionality of c-Abl KD cells was also lower. Furthermore, migration velocity of c-Abl KD cells was also decreased compared with that of uninfected cells and cells expressing control shRNA (Fig. 1, C–G).

We also evaluated effects of the c-Abl pharmacological inhibitor GNF-5 (40) on cell motility. Treatment with GNF-5 reduced cell motility, attenuating the net and total migration paths, directionality, and velocity of smooth muscle cells (Fig. 1, D–G). Moreover, another c-Abl inhibitor imatinib (Gleevec, STI-571) (18) had similar inhibitory effects on cell migration (Fig. 1, D–G).

Adhesion to extracellular matrix promotes cortactin phosphorylation in cells. Cortactin is a tyrosine-phosphorylated protein that has a role in the regulation of actin filament assembly (1, 9). Previous studies have shown that Tyr-421, Tyr-466, and Tyr-482 are phosphorylation sites on mouse cortactin (22). Structural analysis reveals that human cortactin contains Tyr-421 but not Tyr-466 and Tyr-482 (accession no. NM_005231). Thus we determined whether adhesion to extracellular matrix induces cortactin phosphorylation at Tyr-421, an indication of cortactin activation (1, 9), in human smooth muscle cells. HASM cells were plated on collagen-uncoated or coated dishes for 30 min. Cortactin phosphorylation was evaluated by immunoblot analysis. Levels of cortactin phosphorylation in coated surfaces were higher than in uncoated surfaces (Fig. 2A).

Cortactin interacts with Pfn-1. Pfn-1 is an actin-regulatory protein that has a role in regulating cellular functions including smooth muscle contraction and cell motility (11, 30, 32). The interaction of cortactin with Pfn-1 has not been investigated. To determine whether cortactin directly binds to Pfn-1, cortactin immunoprecipitates were immobilized to the membrane, which was reacted with Pfn-1 and detected using Pfn-1 antibody. Pfn-1 was detected in the membrane with immobilized cortactin but not in control membrane (Fig. 2B). Since cortactin undergoes phosphorylation at Tyr-421 in response to adhesion, this raises the possibility that cortactin phosphorylation may modulate its interaction with Pfn-1. To test this, cortactin Y421F mutant immobilized on the membrane was overlaid with Pfn-1, and minor Pfn-1 was found on the immobilized mutant (Fig. 2B).

c-Abl, phospho-cortactin, and Pfn-1 are colocalized in the leading edge. As described earlier, the actin-regulatory proteins cortactin and Pfn-1 have a role in the regulation of cell movement (1, 11). Moreover, cortactin undergoes phosphorylation during cell adhesion. This raises the possibility that phosphorylated cortactin and Pfn-1 may be also involved in the cell edge. To test this, we determined whether these proteins are colocalized in the cell edge by immunofluorescent microscopy. As shown in Fig. 2C, c-Abl, phospho-cortactin, and Pfn-1 were found in the leading edge of cells.

Previous studies have suggested that extracellular matrix facilitates cell adhesion and spreading (5, 10). To verify this, we evaluated morphology of cells on uncoated or coated surfaces. Compared with those on coated surfaces (Fig. 2Db), cells plated on uncoated surface for 30 min (Fig. 2Da) were smaller in size and did not form the leading edge, and phosphorylated cortactin was not found in the cell periphery. Our results confirm previous studies by others (5, 10).

c-Abl mediates cortactin phosphorylation in cells during adhesion and in vitro. We determined whether c-Abl has a role in regulating cortactin in human cells by evaluating the effects of c-Abl KD on cortactin phosphorylation during adhesion using immunoblot analysis. Levels of the adhesion-induced cortactin phosphorylation were reduced in c-Abl KD cells compared with uninfected cells and cells producing scramble shRNA (Fig. 3A).

We also evaluated the effects of c-Abl KD on cortactin phosphorylation in the cell edge by immunostaining. As previously described, the lentiviruses for generating shRNAs also carry green fluorescent protein (GFP) gene to monitor infection efficiency of cells (19, 36). As shown in Fig. 3B, GFP signal was detected in c-Abl KD cells and cells expressing control shRNA, suggesting successful infection of the cells. Moreover, compared with cells expressing control shRNA, localized cortactin phosphorylation was diminished in c-Abl KD cells 30 min after adhesion (Fig. 3, B and C).

We used the in vitro kinase assay to determine the role of c-Abl in cortactin phosphorylation. The addition of purified c-Abl protein resulted in an increase in cortactin phosphorylation at Tyr-421, suggesting direct role for c-Abl in catalyzing cortactin phosphorylation (Fig. 3D).

Pfn-1 localization and F-actin in the cell edge are reduced in c-Abl KD cells. Thus far, we have discovered that c-Abl mediates cortactin phosphorylation, which modulates its interaction with Pfn-1 (Figs. 2B and 3A). We then determined whether c-Abl KD affects Pfn-1 spatial localization in the cell edge. The amount of localized Pfn-1 in the cell edge was reduced in c-Abl KD cells compared with control cells (Fig. 3, B and C).
Fig. 4. Characterization of the cell-permeable peptide CTTN-I. A: blots of cortactin immunoprecipitates (IP) from adherent cells treated with peptides were probed with the use of antibodies against Pfn-1 and cortactin. Ratios of Pfn-1 to cortactin in cells treated with CTTN-I peptide are normalized to the values in cells treated with control peptide. Values represent means ± SE (n = 4). B: blots of adherent cells treated with CTTN-I or control peptides for 30 min were detected with antibodies against phospho-cortactin (Tyr-421) and total cortactin. Phosphorylation levels in cells treated with CTTN-I peptide are normalized to the levels in cells treated with control peptide. Values represent means ± SE (n = 6). C: representative images showing the effects of peptides on phosphorylated cortactin, Pfn-1, and F-actin in the cell edge. Cells were plated on collagen-coated cover-slips in the present of CTTN-I or control peptides for 30 min. Phospho-cortactin and Pfn-1 were evaluated by immunofluorescent microscopy. F-actin was visualized by phalloidin staining. Scale bar, 10 μm. D: quantification analysis of cortactin phosphorylation, Pfn-1, and F-actin in the cell edge. Fluorescence intensity of corresponding proteins in cells treated with CTTN-I peptide is normalized to that in cells treated with control peptide (n = 28–32). *P < 0.05.

Disruption of recruitment of Pfn-1 to cell edge by CTTN-I peptide. To determine whether the association of cortactin with Pfn-1 is important for cell motility, we developed CTTN-I peptide, a cell-permeable peptide, to disrupt the protein-protein interaction. The sequence of this peptide contains Tyr-421 and adjacent residues. We evaluated the effects of this peptide on the spatial distribution of Pfn-1. Coimmunoprecipitation analysis showed that CTTN-I peptide inhibited the interaction of Pfn-1 with cortactin (Fig. 4A). Moreover, immunoblot analysis showed that adhesion-induced cortactin phosphorylation was not affected by CTTN-I peptide (Fig. 4B). More importantly, localized Pfn-1 was reduced in cells treated with CTTN-I peptide compared with cells treated with control peptide. However, phosphorylated cortactin in the cell edge was similar in cells treated with CTTN-I or control peptides (Fig. 4C). Treatment with CTTN-I peptide diminished the fluorescence intensity of Pfn-1 in the cell periphery by 60%, without affecting cortactin phosphorylation (Fig. 4D). Furthermore, treatment with CTTN-I peptide inhibited the intensity of F-actin staining in the cell edge (Fig. 4, C and D). The results suggest that CTTN-I peptide selectively impairs the association of Pfn-1 with cortactin, the recruitment of Pfn-1 to the cell edge, and localized F-actin assembly.

Recruitment of Pfn-1 to leading cell edge is essential for cell migration. We then assessed the effects of CTTN-I peptide on cell motility. Cells were treated with control peptide or CTTN-I peptide, and their motility was evaluated by time-lapse microscopy. The net paths, total paths, velocity, and directionality of cells treated with CTTN-I peptide were diminished compared with those of cells treated with control peptide (Fig. 5, A–D).

Localization of β1-integrin in the cell edge. β1-Integrin is highly expressed in smooth muscle cells/tissues and interacts with α-subunits to form the transmembrane receptors (29, 30). We used immunofluorescent microscopy to assess the spatial local-
Spatial localization of c-Abl is regulated by β1-integrin. To test the hypothesis that β1-integrin may have a role in controlling c-Abl spatial localization, cells were transfected with β1-integrin sense or antisense oligonucleotides for 2 days. Immunoblot analysis verified the downregulation of β1-integrin in cells (Fig. 6, B and C). Furthermore, localized c-Abl distribution was reduced in β1-integrin KD cells compared with that in cells treated with sense oligonucleotides (Fig. 6, D and E).

Localized c-Abl is modulated by actin dynamics. Because actin polymerization has been implicated in mediating intracellular trafficking of the glucose transporter GLUT4 (3), we assessed whether actin dynamics is important for c-Abl localization. Cells were treated with the actin polymerization inhibitor latrunculin A. The spatial distribution of c-Abl was evaluated by immunofluorescent microscopy. Compared with control cells, the size of cells treated with latrunculin A was smaller, suggesting the inhibition of cell spreading. In addition, c-Abl was barely detected in the cell periphery (Fig. 7).

DISCUSSION

The role of c-Abl in smooth muscle cell migration has not been explored before. In this study, c-Abl was localized in smooth muscle cell leading edge. Furthermore, c-Abl was

Fig. 6. β1-Integrin promotes the recruitment of c-Abl in the cell edge. A: representative image illustrating the cellular localization of β1-integrin in normal HASM cells 30 min after adhesion. Scale bar, 10 μm. B: representative immunoblots showing the effects of β1-integrin antisense or sense on protein expression. Blots of cells transfected with β1-integrin sense or antisense for 2 days were probed with the use of antibodies against β1-integrin and GAPDH. C: ratios of β1-integrin to GAPDH in antisense-treated cells are normalized to those in sense-treated cells (n = 6). *P < 0.05. D: representative micrographs illustrating the role of β1-integrin in c-Abl localization. c-Abl is localized in the leading edge of cells treated with β1-integrin sense (a) but is barely detected in cells treated with β1-integrin antisense (b). Arrows indicate the cell edge. E: fluorescence intensity of c-Abl in the periphery of cells treated with antisense is normalized to that in cells treated with sense (n = 26–30). *P < 0.05.

Fig. 7. Actin polymerization modulates spatial distribution of c-Abl. A: representative micrographs illustrating the roles of actin polymerization in c-Abl localization. Cells were treated with or without 1 μM latrunculin-A (LAT-A) for 15 min and then plated on collagen-coated coverslips for 30 min. The spatial localization of c-Abl was assessed by immunofluorescent microscopy. Arrows indicate cell edge. Scale bar, 10 μm. B: fluorescence intensity of c-Abl in the edge of cells treated with latrunculin A is normalized to that in control cells. *P < 0.01, significantly lower intensity in cells treated with latrunculin A than in control cells (n = 25–29).
necessary for smooth muscle cell motility. More importantly, we discovered a novel mechanism, which is that c-Abl regulates cell migration in part by affecting cortactin phosphorylation and recruitment of Pfn-1 to the leading edge. Finally, the recruitment of c-Abl to the leading edge was regulated by β1-integrin and actin dynamics.

c-Abl is a nonreceptor tyrosine kinase that has been implicated in the regulation of actin dynamics, cell adhesion, proliferation, growth, development, and smooth muscle contraction (2, 8, 16, 18, 19, 28, 36). However, the role of c-Abl in cell migration is not well understood. In this study, c-Abl was localized in the leading edge of smooth muscle cells. Furthermore, silencing of c-Abl by RNAi attenuated smooth muscle cell motility as evidenced by time-lapse microscopy. Similarly, inhibition of c-Abl by GFN-5 or imatinib diminished cell motility. These studies suggest a critical role of c-Abl in regulating smooth muscle cell migration.

Cortactin is a tyrosine-phosphorylated protein that has been implicated in the regulation of actin filament assembly. Cortactin may regulate actin polymerization by affecting the functional state of N-WASP, the actin-related protein 2/3 (Arp2/3), and Nck (1, 9, 22). In this study, cell adhesion to extracellular matrix induced cortactin phosphorylation at Tyr-421, an indicator of cortactin activation (1). Cell adhesion may activate the Abl family kinase through the engagement of transmembrane integrins with extracellular matrix proteins (see below).

Cortactin tyrosine phosphorylation may promote its interaction with the SH2-containing proteins such as Arg and Nck, which in turn activate N-WASP and actin dynamics during cell spreading and migration (1, 9, 22). Here, we discover a new cortactin-interacting protein, Pfn-1. Cortactin is able to bind to Pfn-1 in vitro. Pfn-1 is an actin-regulatory protein that is capable of modulating actin dynamics and cell motility. Pfn-1 promotes actin polymerization by facilitating the transport of G-actin to F-actin (11, 12, 30). Previous studies by others have suggested that the NH2-terminal and COOH-terminal helices of Pfn-1 form a binding cleft for poly-L-proline domain of proteins such as palladin (6, 38). Thus it is likely that cortactin binds to Pfn-1 via the interaction of the proline-rich domain of cortactin with the helices of Pfn-1. Structural analysis reveals that Tyr-421 is located in the proline-rich domain of cortactin (1, 9). To determine whether cortactin phosphorylation affects its interaction with Pfn-1, we evaluated the effects of nonphosphorylatable cortactin mutant on the protein-protein interaction. Cortactin Y421F mutant lost its affinity to Pfn-1. These results suggest that the interaction of cortactin with Pfn-1 is regulated by phosphorylation at Tyr-421. Therefore, Tyr-421 phosphorylation on cortactin may alter conformation of the proline-rich domain and increase the affinity of cortactin to Pfn-1. Since phosphorylated cortactin increased its affinity to Pfn-1, and phospho-cortactin and Pfn-1 were localized in the cell edge, we propose that the recruitment of Pfn-1 to phosphorylated cortactin may promote the activation of Pfn-1 during cell migration.

In mouse embryonic fibroblasts, cortactin phosphorylation is mediated by Src family kinases or Arg (1, 9, 22). In this study, knockdown of c-Abl attenuated the matrix-induced cortactin phosphorylation, demonstrating the important role of c-Abl in regulating cortactin phosphorylation during cell adhesion to matrix. Furthermore, cortactin can be phosphorylated by c-Abl in vitro. Thus c-Abl may directly catalyze cortactin phosphorylation during cell migration.

In myeloid and cancer cells, c-Abl may regulate cell motility by modulating the small GTPases Cdc42 and Rac (4, 21), which may in turn activate N-WASP (1). In addition, Dok-1 and Abi1 may be regulated by c-Abl (37, 39). In this study, KD of c-Abl in cells attenuated cortactin phosphorylation and the recruitment of Pfn-1 to the cell edge. Furthermore, leading edge formation and cell motility were reduced by c-Abl KD. To the best of our knowledge, this is the first evidence to suggest that c-Abl may regulate cell motility in part by modulating cortactin phosphorylation and the recruitment of Pfn-1 to the cell edge.

To determine whether the recruitment of Pfn-1 to the cell edge is involved in the regulation of cell movement, we developed the cell-permeable peptide CTTN-I. Treatment with the peptide inhibited the interaction of Pfn-1 with cortactin but not cortactin phosphorylation. Furthermore, treatment with the peptide attenuated the recruitment Pfn-1 to cortactin and cell motility. These findings are consistent with the hypothesis that the recruitment of Pfn-1 to cortactin in the cell periphery plays a critical role in the regulation of cell migration.

β1-Integrin is a key component of the transmembrane receptors that connect the actin cytoskeleton with extracellular matrix (29, 30). In this study, β1-integrin was also found in the leading cell edge. Furthermore, β1-integrin knockdown impaired the localization of c-Abl in the cell periphery. These results indicate that β1-integrin is necessary for c-Abl recruitment to the leading edge during migration.

The actin cytoskeleton has been implicated in mediating GLUT4 translocation (3). Our results in this study suggest that actin polymerization contributes to the regulation of c-Abl recruitment. Since the COOH terminus of c-Abl has an F-actin binding domain (34), it is likely F-actin in the cell edge may strengthen the anchor of c-Abl to the region.

In this study, we propose a novel mechanism that regulates smooth muscle cell migration. In response to extracellular cues (e.g., matrix protein), c-Abl is activated via interaction with β1-integrin, which in turn mediates cortactin phosphorylation.
Phosphorylated cortactin may recruit Pfn-1 to the cell edge, which promotes localized actin filament rearrangement, leading edge formation, and cell migration. Conversely, actin cytoskeleton remodeling facilitates the recruitment of c-Abl to the cell edge (Fig. 8).

REFERENCES


