A Role for p38MAPK/HSP27 Pathway in Smooth Muscle Cell Migration*

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Smooth muscle cells are exposed to growth factors and cytokines that contribute to pathological states including airway hyperresponsiveness, atherosclerosis, angiogenesis, smooth muscle hypertrophy, and hyperplasia. A common feature of several of these conditions is migration of smooth muscle beyond the initial boundary of the organ. Signal transduction pathways activated by extracellular signals that instigate migration are mostly undefined in smooth muscles. We measured migration of cultured tracheal myocytes in response to platelet-derived growth factor, interleukin-1β, and transforming growth factor-β. Cellular migration was blocked by SB203580, an inhibitor of p38MAPK. Time course experiments demonstrated increased phosphorylation of p38MAPK. Activation of p38MAPK resulted in the phosphorylation of HSP27 (heat shock protein 27), which may modulate F-actin polymerization. Inhibition of p38MAPK activity inhibited phosphorylation of HSP27. Adenovirus-mediated expression of activated mutant MAPK kinase 6b(E), an upstream activator for p38MAPK, increased cell migration, whereas overexpression of p38α MAPK dominant negative mutant and an HSP27 phosphorylation mutant blocked cell migration completely. The results indicate that activation of the p38MAPK pathway by growth factors and proinflammatory cytokines regulates smooth muscle cell migration and may contribute to pathological states involving smooth muscle dysfunction.

Smooth muscle cells are exposed to numerous growth factors and proinflammatory cytokines that contribute to atherosclerosis, angiogenesis, smooth muscle hypertrophy, and hyperplasia and airway hyperresponsiveness during asthma (1–3). Evidence for critical roles of vascular smooth muscle cell migration has been suggested by the finding of cell clonality in lesions of atherosclerosis in postangioplasty restenosis remodeling and vascular smooth cell recruitment in angiogenesis (2, 4). In the respiratory system, recent studies have reported increased concentrations of several growth factors and cytokines in the bronchoalveolar lavage fluid isolated from allergic asthmatic individuals (5). Postmortem studies have further indicated that over time, airway remodeling results from a thickening of the airway wall (6). Hypertrophy and hyperplasia of airway smooth muscle narrows the airway opening leading to increased resistance to airflow and more work required for breathing (7). Many of these growth factors and cytokines such as platelet-derived growth factor (PDGF), interleukin-1β (IL-1β), and transforming growth factor-β (TGFβ) have been identified, but their signaling pathways are not well defined. An understanding of the signal transduction pathways contributing to smooth muscle remodeling and dysfunction will be useful in examining the underlying causes of numerous diseases.

The mitogen-activated protein kinases (MAPKs) have been shown to play an important role in transducing extracellular signals into cellular responses (8, 9). Specific MAPK cascades (MAPKKK → MAPKK → MAPK) are stimulated by a variety of signals including growth factors, cytokines, UV light, and other stress-inducing agents. MAPKs are believed to play a pivotal role in cell proliferation, apoptosis, differentiation, cytoskeletal remodeling, and the cell cycle (10–15). These kinases can be categorized by the sequence of the activating canonical dual phosphorylation site threonine-Xaa-tyrosine (TXY) (16). Current evidence suggests mammalian cells express at least three groups of MAPKs: extracellular signal-regulating kinases (ERK; where Xaa = Glu), p38MAPKs (where Xaa = Gly), and c-Jun N-terminal (where Xaa = Pro) kinases (17, 18–20). It was first demonstrated in monocytes that p38MAPK is activated by bacterial lipopolysaccharide and the proinflammatory cytokines IL-1β and tumor necrosis factor-α (18, 22). Recent reports have demonstrated that other cytokines, growth factors and autonomic neurotransmitters activate p38MAPKs (13, 23, 24). In the family of p38MAPKs, at least four isoforms have been identified (18, 25–27). Experiments have demonstrated that p38MAPK lies downstream of the RAS-related GTP-binding proteins Rac and Cdc42 and is directly activated by kinases, MKK3, MKK4, and MKK6 (19, 20, 28–33).

p38MAPK phosphorylates and activates several transcription factors including ATF-2, CHOP, ELK-1, Saplα, and MEF2C (25, 32, 34–36). p38MAPK also phosphorylates and activates downstream protein kinases, MAPKAP kinase-2, MAPKAP kinase-3, and p38-regulated/activated protein kinase (37–39). Several experiments have indicated that the small heat shock protein, HSP27, is a physiological substrate for these kinases. The phosphorylation of three serine residues on HSP27 ap-
pears to modulate the polymerization of actin and is proposed to play a role in actin, cytoskeleton remodeling during cellular stress, and growth (40).

Multiple protein systems such as actin, myosins, and microtubules are involved in cytoskeleton remodeling and cell migration. Although much is known about regulation of smooth muscle myosin by phosphorylation, less is known about remodeling of smooth muscle actin. It seems likely that many of the extracellular signals that stimulate actin remodeling in non-muscle cells would do so in smooth muscles. However, little is known about the signal transduction pathways coupling cytoskeleton and growth factors receptors to proteins that regulate actin remodeling in smooth muscle cells. In this report, a cell migration assay was used as an indirect measure of functional effects of actin cytoskeleton remodeling. We demonstrate that tracheal smooth muscle cells migrate in response to PDGF, IL-1β, and TGFβ, and we present data showing that these chemical mediators activate the p38MAPK pathway leading to the phosphorylation of HSP27. We also demonstrate that cellular migration is blocked by the p38MAPK specific inhibitor, SB203580 (22), and by overexpression of p38α MAPK dominant negative mutant and an HSP27 phosphorylation mutant. Furthermore, an upstream activator for p38MAPK, activated mutant MAPK kinase 6b(E) (MKK6bE) increased cell migration. Taken together, these results indicate that activation of p38MAPK pathway by proinflammatory cytokines and growth factors modulates smooth muscle migration and remodeling.

EXPERIMENTAL PROCEDURES

Materials—Adult mongrel dogs of either sex were sacrificed by barbiturate overdose. The trachea was removed and placed in cold physiological salt solution composed of 2 mM MOPS, pH 7.4, 140 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO₄, 2.5 mM CaCl₂, 1.2 mM Na₂HPO₄, 0.02 mM ethylenediaminetetraacetate, and 5.6 mM D-glucose. [32P] was purchased from ICN Biomedicals, Inc. Phospho-specific p38MAPK antibodies were purchased from New England Biolabs (Beverly, MA). p38MAPK and MKK6 antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-FLAG-tagged antibodies were purchased from Eastman Kodak Co. Anti-hemagglutinin-tagged antibodies were purchased from Roche Molecular Biochemicals. Anti-human HSP27 was purchased from StressGen Biotechnologies Corp. (Victoria, BC, Canada). Anti-canine HSP27 has been previously described (41). Anti-rabbit and anti-mouse IgG alkaline phosphatase conjugate antibodies were purchased from Promega Corp. (Madison, WI). SB203580 and PD98059 were purchased from Calbiochem (La Jolla, CA).

Cell Migration Assay—Cell migration was assayed using a modified Boyden chamber assay as described previously (24). Tracheal smooth muscle cells were dispersed using collagenase (0.6 mg/ml) and grown to confluence in M-199 culture medium (Life Technologies, Inc.) containing 10% fetal bovine serum. At confluence, cells were placed in serum-free M-199 for 24 h prior to migration experiments. Smooth muscle cells were harvested with trypsin (0.1 mg/ml trypsin), counted, centrifuged, and resuspended at 8.0 × 10⁴ cell/ml in 0.3% BSA M-199 medium (Life Technologies, Inc.). Cells were plated on the upper side of a collagen-treated, polycarbonate membrane (8.0 μm pore) separating two chambers of a 6.5-mm transwell culture plate (Costar). Cells were diluted to 0.3% BSA M-199 as a negative control (upper and lower chamber), or PDGF, IL-1β, or TGFβ (lower chamber) was added. SB203580 (25 μM), PD98059 (25 μM), or the vehicle (0.1% Me₂SO) was added to both chambers 30 min before treatments. After 5 h, cells on the upper face of the membrane were scraped using a cotton swab. Cells that migrated to the lower face of the membrane were fixed with 3.7% formaldehyde and stained with DifQuik (Baxter Scientific Products) Wright-Giemsa solution. The number of migrated cells on the lower face of the filter was counted in five fields under 10× magnification. Assays were done in duplicate and were repeated five times.
times using cells from different animals.

**p38MAPK Phosphorylation in Airway Smooth Muscle Cells**—Tracheal smooth muscle cells were grown on 6-well plates as described above for the cell migration experiments. After 24 h in serum-free medium, cells were stimulated with PDGF (10 ng/ml), IL-1β (6 ng/ml), and TGFβ (1 ng/ml) for 0, 1, 5, 10, 20, and 60 min. Cells were lysed, and proteins were extracted in SDS-PAGE sample buffer (see "Experimental Procedures"). Total proteins were resolved by SDS-PAGE and tyrosine/threonine phosphorylation of p38MAPK detected by Western blotting with anti-phospho-tyrosine/threonine-p38 MAPK antibody and alkaline phosphatase conjugated secondary antibody. Images of immunoblots (upper panels) illustrate relative levels of p38MAPK tyrosine phosphorylation. Relative phosphorylation was determined by scanning densitometry and is presented as the means ± S.E. in the bar graphs below each blot image. n = 5, *p < 0.05 versus control.

**Fig. 2.** Phosphorylation of p38MAPK in stimulated tracheal smooth muscle cells. Cells from five experiments were stimulated with 200 μM sodium arsenite (A), PDGF (10 ng/ml) (B), IL-1β (6 ng/ml) (C), and TGFβ (1 ng/ml) (D) for 0, 1, 5, 10, 20, and 60 min. Cells were lysed, and proteins were extracted in SDS-PAGE sample buffer (see "Experimental Procedures"). Total proteins were resolved by SDS-PAGE and tyrosine/threonine phosphorylation of p38MAPK detected by Western blotting with anti-phospho-tyrosine/threonine-p38 MAPK antibody and alkaline phosphatase conjugated secondary antibody. Images of immunoblots (upper panels) illustrate relative levels of p38MAPK tyrosine phosphorylation. Relative phosphorylation was determined by scanning densitometry and is presented as the means ± S.E. in the bar graphs below each blot image. n = 5, *p < 0.05 versus control.

**Recombinant Adenovirus Vectors and Cell Infections**—Adenoviruses expressing activated MKK6E and the p38δ MAPK dominant negative (TGY → AGF) mutant (p38δdn) were a generous gift from Dr. Yibin Wang (University of Maryland) and have been described previously (42). Ad-3A and Ad-WT are replication-defective adenoviral recomb-
nultants prepared as described previously (43). Ad-3A contains cDNA for mutant human HSP27 (Ad-3A). Ad-WT contains an insert coding for wild type human HSP27. Expression of the transgenes are driven by a cytomegalovirus (CMV)-promoter inserted in the E1 region of E1 deleted human adenovirus type 5. Ad-R is an empty control vector. Mutant HSP27 differs from the wild type in that the three known phosphorylation sites (Ser-15, Ser-78, and Ser-82) were mutated to alanines (44). It has been previously demonstrated that mutation of these sites to nonphosphorylatable amino acids (glycines) results in a phosphorylation deficient mutant (45, 46). The triple alanine mutant was made using the Muta Gene Kit (Bio-Rad). The glycine triple mutant (45) was subcloned into M13 mp19 and grown in E. coli strain CJ237. Primers were obtained to change codon 15 to ala mutation. The subcloned, mutagenized gene fragment was completely sequenced to verify the intended mutation, or the codon15 to ala mutation. The subcloned, mutagenized gene fragment was completely sequenced to verify the intended mutation and to exclude the possibility of other sequences being altered during the mutagenesis protocol. Restriction fragments were ligated into the hsp27 gene in the plasmid Bluescript KS to reconstruct a clone containing all three serine-alanine codon changes. The final clone was prepared from phage containing either the codon 78 to ala mutation, or the codon15 to ala mutation. The subcloned, mutagenized clone was completely sequenced to verify the intended mutation and to exclude the possibility of other sequences being altered during the mutagenesis protocol. Restriction fragments were ligated into the hsp27 gene in the plasmid Bluescript KS to reconstruct a clone containing all three serine-alanine codon changes. The final clone was verified by double strand sequencing. Adenovirus vectors were produced and purified from the 293 packaging cell line (Microbiz, Toronto, ON, Canada) as described previously (47). Cells were infected with Ad vectors at a multiplicity of infection of 20 plaque-forming units/cell. Medium was removed, and 1 × 10⁵ cells/well were infected with 200 µl of diluted virus in phosphate-buffered saline for 60 min. The cells were incubated in 2 ml/well of low serum (0.1% newborn calf serum) for 4–7 days. Cells were then used in the cell migration assay as described above or for biochemical assays. Transduction efficiency (greater than 95%) and transgene expression were verified by immunoblotting and immunofluorescence.

**HSP27 Phosphorylation**—One-dimensional isoelectric focusing (IEF) was used to separate nonphosphorylated and phosphorylated isoforms of HSP27. Proteins were extracted in SDS sample buffer (60 mM Tris-HCl, pH 6.8, 2% SDS, 1% glycerol, 1 mM leupeptin, 10 mM EGTA, 1 mM Na3EDTA, 1 mM p-aminophenylbenzenesulfonyl fluoride HCl, and 5 mM NaF). Samples were diluted in an equal volume of IEF buffer (9.0 M urea, 2% Chaps, 2% ampholines (75% Bio-lyte 5–7, 25% Bio-lyte 3–10, 5% β-mercaptoethanol, 10 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 1 mM EDTA). IEF was carried out on tube gels as described previously (48). IEF tube gels were equilibrated in SDS sample buffer for 1 h at 37°C. Proteins were transferred to nitrocellulose as described above and were probed with a canine HSP27 anti-serum (1:5000) or an anti-human HSP27 monoclonal antibody (1:12000) followed by goat anti-rabbit-IgG or anti-mouse-IgG alkaline phosphatase secondary antibody. Images of immunoblots were analyzed as described above. Densitometric data of HSP27 isoforms were expressed as percentages of total HSP27. Validity of this technique is based on our previous study describing phosphorylation of charge isoforms of HSP27 in tracheal smooth muscle (41).

**Statistical Analysis**—Analysis between two groups was performed using unpaired two-tailed t tests, where p values less than 0.05 are considered significantly different.

**RESULTS**

**Smooth Muscle Cell Migration in Response to PDGF, IL-1β, and TGFβ**—Smooth muscle cells are exposed to numerous growth factors and proinflammatory cytokines that contribute to smooth muscle remodeling during asthma, atherosclerosis, and angiogenesis (1–3). Some of these growth factors and cytokines such as PDGF, IL-1β, and TGFβ have been identified, but their signaling pathways are not well defined. In many of these disease states smooth muscle cells migrate and begin to proliferate. Cell migration depends on the remodeling of cytoskeletal proteins such as actin and myosin, and it has been shown that blocking actin remodeling inhibits cell motility (24).
Moreover, phosphorylation of HSP27 modulates actin remodeling (45), and phosphorylation of HSP27 is regulated by the p38MAPK/MAPKAP-2/3 pathway (48). To test the hypothesis that a decrease in p38 MAPK signaling and phosphorylation of HSP27 would inhibit cell motility, we assayed cell migration using a modified Boyden chamber assay (49). We plated tracheal smooth muscle cells on collagen-coated polycarbonate upper membranes and added PDGF, IL-1β, and TGFβ to the lower chamber of a transwell culture plate. Cell migration was assayed after 5 h, and the results are presented in Fig. 1. PDGF (1–10 ng/ml) and the proinflammatory cytokine, IL-1β (1–6 ng/ml) stimulated a concentration-dependent increase in cell migration with greater than 8- and 6-fold increases in migration compared with control cells, respectively (Fig. 1). TGFβ (1–5 ng/ml) also stimulated an increase in cell migration at an optimal concentration of 1 ng/ml, whereas higher concentrations seem to be inhibitory (Fig. 1B).

To test the notion that the p38MAPK pathway has a role in cell migration induced by cytokines and growth factors, we pretreated tracheal myocytes with SB203580. SB203580 is a pyridinyl imidazole inhibitor of p38α and p38β MAPK isoforms that, as we have shown, blocks p38MAPK activation and HSP27 phosphorylation with no effect on the ERK MAPKs in tracheal smooth muscle (41). Cellular migration was blocked after myocytes were pretreated with 25 μM SB203580 for 30 min (Fig. 1). Cell migration was reduced nearly to that of the unstimulated control cells. Because it has been reported that PDGF activates the ERK1/2 pathway, we tested the notion that ERK1/2 also has a significant role in PDGF-stimulated smooth muscle cell migration. We pretreated cells with 25 μM PD98059, a compound that specifically inhibits the ERK1/2 kinase MEK (50). Cell migration was blocked about 15% in myocytes treated with 25 μM PD98059 (Fig. 1A). Phosphorylation of p38 MAPK in Stimulated Smooth Muscle Cells—p38MAPK is activated by upstream kinases MKK3 and MKK6 by dual phosphorylation of threonine 180 and tyrosine 182 in the regulatory TGY motif (16). Phosphorylation of this motif has been used as an index of p38MAPK activation and can be assayed with an anti-p38 MAPK phospho-tyrosine/threonine-specific antibody recognizing the phosphorylated TGY motif (16, 23). To test the notion that p38MAPK is activated by PDGF, IL-1β, and TGFβ, tracheal smooth muscle cells were treated for

**Fig. 4.** *In vivo* phosphorylation of HSP27. Tracheal smooth muscle cells were labeled with H$_3^32$P(O$_4$) (25 μCi/ml) for 2 h. Cells were left untreated or were treated with 200 μM sodium arsenite (A), PDGF (10 ng/ml) (B), IL-1β (6 ng/ml) (C), and TGFβ (1 ng/ml) (D) for 30 min. Treated cells were pretreated with (+) and without (−) 25 μM SB203580. Phosphoproteins were resolved by SDS-PAGE, and radioactive phosphorous incorporation was measured by imaging gels with a Bio-Rad Molecular Imager. These data are representative of at least two independent experiments.

**Fig. 5.** Adenoviral-mediated over-expression of p38MAPK signaling molecules in airway myocytes. A, airway myocytes were infected with replication-defective adenovirus expressing β-galactosidase at a multiplicity of infection of 0 (panel a) and 20 (panel b) viral particles/cell. Greater than 95% of the myocytes expressed the transgene after 96 h. Cells were photographed under phase contrast microscopy in a light field. B, protein extracts from 80,000 airway myocytes were analyzed by Western blot to detect expression of MKK6bE and p38adn using anti-hemagglutinin and anti-FLAG monoclonal antibodies, respectively. Cells were also infected with a control virus (Ad-R) lacking a transgene insert. C, p38MAPK phosphorylation levels were measured by Western blot analysis. D, p38MAPK activity was measured using ATF-2 as a substrate. Uninfected cells, cells infected with a control virus, MKK6bE, and p38adn were stimulated with (+) or without (−) 10 ng/ml PDGF for 30 min. Cellular extracts were used to phosphorylate 1 μg of ATF-2 in vitro for 30 min. Phosphoproteins were resolved by SDS-PAGE, and radioactive phosphorous incorporation was measured by imaging gels with a Bio-Rad Molecular Imager.
Expression of \( p38^{\text{MAPK}} \) Signaling Molecules in Myocytes by Adenovirus Vectors—To further study the role of the \( p38^{\text{MAPK}} \) pathway in smooth muscle cell migration, we utilized recombinant adenoviruses to overexpress a constitutively activated mutant upstream activator of \( p38^{\text{MAPK}}, \) MKK6bE, and a dominant negative mutant of \( p38 \alpha \) MAPK isoform, \( p38\text{dn} \) (42). As demonstrated in Fig. 5A with an adenovirus vector expressing \( \beta \)-galactosidase, greater than 95% of the myocytes were transduced when infected with an multiplicity of infection of 20 after 96 h. Transgene expression levels were detected by Western blot analysis (Fig. 5B). Untreated MKK6bE-infected myocytes demonstrated an increased level of \( p38^{\text{MAPK}} \) activation (Fig. 5, C and D), whereas treated \( p38\text{dn} \)-infected cells showed decreased \( p38^{\text{MAPK}} \) activity (Fig. 5D).

Activation of the \( p38^{\text{MAPK}} \) Pathway Induces Airway Myocyte Cell Migration—To test the hypothesis that \( p38^{\text{MAPK}} \) regulates smooth muscle cell migration we overexpressed an activated \( p38^{\text{MAPK}} \) upstream activator, MKK6bE. Smooth muscle cells that overexpressed MKK6bE increased cell migration in both the presence and absence of PDGF (Fig. 6). In the presence of PDGF, MKK6bE-infected cells migrated to a greater than 40% compared with uninfected cells or infected with a control virus. Even without the addition of PDGF, the MKK6bE-infected cells migrated 2-fold greater than the control or uninfected cells. However, cell migration was completely abolished in cells that were overexpressing the \( p38 \alpha \) MAPK dominant negative isoform, which was consistent with the results of the SB203580-treated cells in Fig. 1A. Moreover, fewer \( p38\text{dn} \)-infected cells migrated both in the presence and absence of agonist compared with control and uninfected cells without agonist.

Phosphorylation Mutant HSP27 Inhibits Cell Migration—It has been determined that HSP27 is phosphorylated by MAPKAP-2/3 on three serine amino acids. To test for a role of HSP27 in cell migration more directly, we expressed HSP27 phosphorylation mutant in cultured myocytes. This strategy of using an HSP27 phosphorylation mutant has been shown previously to inhibit F-actin formation (46). The HSP27 phosphorylation mutant was constructed by mutating three serine residues (Ser-15, Ser-78, and Ser-82) to alanines. Tracheal smooth muscle cells were infected with adenovirus vectors encoding a human HSP27 mutant cDNA (Ad-3A), a wild type human HSP27 (Ad-WT) and a control vector lacking an insert (Ad-R). These cells were then plated and used in the cell migration assay as described above. The cells were treated with 10 ng/ml of PDGF to stimulate maximal cell migration, and the results from five experiments are presented in Fig. 7. The same increase in cell migration was observed with the control vector, wild type human HSP27, and in uninfected cells in the presence of PDGF (Fig. 7). Cell migration was inhibited in myocytes by expressing the HSP27 phosphorylation mutant transgene. Western blot analysis demonstrated similar expression levels (50 ng/\( \mu \text{g of total protein} \)) for both the mutant and wild type transgenes (Fig. 8A). The levels of endogenous canine HSP27 (8 ng/\( \mu \text{g of total protein} \)) were unaffected by infection of the virus or expression of the transgenes (Fig. 8A). Expression of the human wild type and mutant HSP27 did not inhibit activation of \( p38^{\text{MAPK}} \) (Fig. 8B) or HSP27 activation (Fig. 8C) by PDGF, nor did it inhibit phosphorylation of the endogenous canine HSP27 (Fig. 9) or the human HSP27 (data not shown). HSP27 isoforms A, B, C, and D correspond to unphosphorylated and mono-, di-, and tri-phosphorylated HSP27, respectively. Cells that were infected with the adenovirus vectors contained a higher percentage of phosphorylated HSP27 isoforms in the...
absence of cytokine stimulation than the uninfected control cells (Fig. 9, A–D). However, adenovirus infection and expression of the transgene did not inhibit isoform shifting in treated cells, suggesting signal transduction between the receptor and activation of MAPKAP-2/3 is unaffected.

**DISCUSSION**

We show that PDGF, TGFβ, and the proinflammatory cytokine, IL-1β, activate the p38MAPK pathway and mediate cell migration in smooth muscle cells. Smooth muscle cells are exposed to numerous growth factors and proinflammatory cytokines that contribute to the pathogenesis of many airway and vascular diseases. Previous studies have further indicated that over time, airway smooth muscle remodeling results from a thickening of the airway wall because of hyperplasia and hypertrophy of airway smooth muscle (6). A current hypothesis is that these chemical mediators are responsible for smooth muscle remodeling and hyperresponsiveness by affecting smooth muscle growth, cytokine and matrix biosynthesis, and actin cytoskeleton remodeling. Using a cell migration assay, which depends in part on actin remodeling, we demonstrated that PDGF, IL-1β, and TGFβ induce cell migration in tracheal myocytes (Fig. 1). PDGF and IL-1β stimulated a concentration-dependent increase in cell migration. TGFβ (1 ng/ml) stimulated migration at least 2-fold over the Me 2SO control. A recent report demonstrated that p38 MAPK activation by vascular endothelial growth factor-mediated cell migration and actin reorganization in human endothelial cells (24). To test our hypothesis that PDGF, IL-1β, and TGFβ induce cell migration in tracheal myocytes (Fig. 1), PDGF and IL-1β stimulated a concentration-dependent increase in cell migration. TGFβ (1 ng/ml) stimulated migration at least 2-fold over the Me 2SO control. A recent report demonstrated that p38MAPK activation by vascular endothelial growth factor-mediated cell migration and actin reorganization in human endothelial cells (24). To test our hypothesis that PDGF, IL-1β, and TGFβ induce cell migration in tracheal myocytes, we overexpressed an activated p38 MAPK kinase mutant, MKK6bE, a p38α MAPK dominant negative mutant, and we pretreated airway myocytes with the p38 MAPK inhibitor, SB203580 (22). Cell migration was blocked when myocytes were pretreated with 25 μM SB203580 for 30 min (Fig. 1) and in cells expressing p38αdn (Fig. 6). Cell migration was increased in cells expressing MKK6bE both in the presence and absence of agonist. The results of the cell migration experiments suggest that p38MAPK activation stimulates cell migration, possibly by regulating actin remodeling. To test this notion, we treated tracheal smooth muscle cells with concentrations of PDGF, IL-1β, and TGFβ that resulted in maximal migration. Time course experiments demonstrated increased tyrosine and threonine phosphorylation of p38MAPK by all agonists (Fig. 2).
The mechanisms by which p38MAPK modulates actin cytoskeleton remodeling in response to PDGF, IL-1β, and TGFβ remain to be determined. In many cell types, MAPKAP-2/3 are not inhibited in infected cells. However, it appears that expression of the wild type HSP27 transgene does not block phosphorylation of the endogenous HSP27 (Fig. 7). One possible explanation for inhibition of cell migration in myocytes overexpressing the HSP27 phosphorylation mutant is that the transgene is interfering with upstream signaling. To test this possibility, we included control experiments showing that upstream signaling is not interrupted by overexpression of the HSP27 mutant (Fig. 6, B and C). Activation of p38MAPK and MAPKAP-2/3 were not inhibited in infected cells.

In summary, this study demonstrates that activation of the p38MAPK/HSP27 pathway is involved not only in cellular response to stress but also in physiological signaling of smooth muscle cells. Using a cell migration assay, biochemical kinase assays, and adenovirus-mediated overexpression of a phosphorylation mutant HSP27, we were able to demonstrate a role for activation of p38MAPK and HSP27 phosphorylation in regulating tracheal smooth muscle cell migration in response to growth factors and proinflammatory cytokines.

Smooth muscle cell migration has been suggested to contribute to pathology in lesions of atherosclerosis, in postangioplasty restenosis remodeling and vascular smooth cell recruitment in angiogenesis, in airway remodeling in asthmatics, and in smooth muscle tumors such as uterine leiomyomas. (2, 4, 21). An understanding of the signal transduction pathways that regulate smooth muscle cell migration may contribute to development of novel therapeutic strategies to inhibit the role of smooth muscle cell migration in diseases.

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FIG. 9. Expression of human HSP27 transgenes does not inhibit in vivo phosphorylation of endogenous HSP27. Tracheal smooth muscle cells not infected (NI), infected with control virus (Ad-R), HSP27 phosphorylation mutant (Ad-3A), and human wild type HSP27 (Ad-WT) were stimulated with PDGF (10 ng/ml) for 30 min. Endogenous HSP27 phosphorylation isoforms (lanes A-D) were resolved by one-dimensional IEF gels and assayed by Western analysis. Isoforms A, B, C, and D correspond to unphosphorylated and mono-, di-, and triphosphorylated HSP27 respectively. Results from unstimulated and stimulated cells are presented as the percentage of total HSP27 isoforms. These data are representative of at least two independent experiments.
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