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 initiate 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 muscle 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, cytoskeleton 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, M KK3, M KK4, and M KK6 (19, 20, 28–33).

p38MAPK phosphorylates and activates several transcription factors including ATF-2, CHOP, ELK-1, Sap1a, 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 cytoskie and growth factor 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. We also 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 dispensed 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 × 10⁵ 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 in 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) diluted in 0.3% BSA M-199 medium were 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.

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. 

p38MAPK Mediates Cell Migration

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**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.
Fig. 3. HSP27 activation kinetics. Cells 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, 60, 90, and 120 min. Cells were also pretreated with 25 μM SB203580 for 30 min and then lysed after 20 min stimulation. Protein extracts were used to phosphorylate rHSP27 in vitro. The kinase reaction was stopped by addition of SDS-PAGE sample buffer after 30 min. Phosphoproteins were resolved by SDS-PAGE, and radioactive phosphorous incorporation was measured by imaging gels with a Bio-Rad Molecular Imager. 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 cells treated for 20 min without inhibitor.

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. 1A). TGFβ (1–5 ng/ml) also stimulated an increase in cell migration at an optimal concentration of 1 ng/ml, whereas higher concentrations seemed 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).

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...
various periods of time with concentrations that induced maximal cell migration. Time course experiments, presented in Fig. 2, demonstrate a transient increase in tyrosine and threonine phosphorylation of p38MAPK. Stimulation with PDGF (10 ng/ml) induced a 7-fold increase in p38MAPK phosphorylation after 20 min (Fig. 2B). There was a 6-fold increase in p38MAPK phosphorylation in response to 6 ng/ml of IL-1β (Fig. 2C) and a 4-fold increase in phosphorylation with TGFβ (1 ng/ml) (Fig. 2D) after 20 min. As a positive control for the activation of p38MAPK, cells were also treated with 200 μM sodium arsenite for the same time periods (Fig. 2A).

**HSP27 Activation Kinetics—**MAPKAP kinase-2 and -3 are potential p38MAPK effector proteins that phosphorylate HSP27 in vitro and in vivo (41). Recently, a new p38MAPK effector protein, p38-regulated/activated protein kinase, has been identified that phosphorylates HSP27; however, it has not been determined whether p38-regulated/activated protein kinase is expressed in smooth muscle cells (37). Using an in vitro assay for HSP27 activation as described previously (41), we determined the activation kinetics of HSP27 activation in cells treated as described above. Cellular extracts were used to phosphorylate rHSP27 in vitro. The kinase reaction was added by addition of concentrated SDS-PAGE sample buffer, and the phosphoproteins were resolved by SDS-PAGE. Radioactive phosphor incorporation was measured by imaging dried gels with a Bio-Rad Molecular Imager. Results presented in Fig. 3 demonstrate that HSP27 activation followed activation kinetics similar to those of p38MAPK phosphorylation. Time course experiments demonstrated a transient increase in activation. Stimulation with PDGF induced a maximal activation after 10 min (Fig. 3B). Maximal activation in response to IL-1β (Fig. 3C), TGFβ (Fig. 3D), and sodium arsenite (Fig. 3A) were observed at 20 min. HSP27 activation was also blocked in cells that were pretreated with 25 μM SB203580 (Fig. 3, 20 min). These results argue that p38MAPK is responsible for activation of HSP27 in cells treated with PDGF, IL-1β, and TGFβ.

**Phosphorylation of HSP27—**The phosphorylation of HSP27 appears to modulate the polymerization of actin (40) and is proposed to play a role in actin cytoskeleton remodeling. To determine that PDGF, IL-1β, and TGFβ induce phosphorylation of HSP27 in vitro through the p38MAPK pathway in migrating cells, we labeled cells using H3[32P]O4. Results presented in Fig. 4 demonstrate that HSP27 is phosphorylated in treated cells after 30 min. Moreover, pretreatment with 25 μM SB203580 blocked phosphorylation of HSP27 in all treated cells.

**Expression of p38MAPK Signaling Molecules in Myocytes by Adenovirus Vectors—**To further study the role of the p38MAPK pathway in smooth muscle cell migration, we utilized recombinant adenoviruses to overexpress a constitutively activated mutant upstream activator of p38MAPK, MKK6bE, and a dominant negative mutant of p38α MAPK isoform, p38dn (42). As demonstrated in Fig. 5A with an adenovirus vector expressing β-galactosidase, greater than 95% of the myocytes were transduced when infected with a 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 p38MAPK activation (Fig. 5, C and D), whereas treated p38dn-infected cells showed decreased p38MAPK activity (Fig. 5D).

**Activation of the p38MAPK Pathway Induces Airway Myocyte Cell Migration—**To test the hypothesis that p38MAPK pathway regulates smooth muscle cell migration we overexpressed an activated p38MAPK 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 p38α MAPK dominant negative isoform, which was consistent with the results of the SB203580-treated cells in Fig. 1A. Moreover, fewer p38dn-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 an 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/μg of total protein) for both the mutant and wild type transgenes (Fig. 8A). The levels of endogenous canine HSP27 (8 ng/μg 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 p38MAPK (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 induced 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 were activating the p38MAPK pathway, we overexpressed an activated p38 MAPK kinase mutant, MKK6bE, a p38α MAPK dominant negative mutant, and we pretreated airway myocytes with the p38MAPK 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).
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 tri-phosphorylated 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.

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 is an identified p38MAPK substrate. Kinase activity for HSP27 was also activated in treated airway smooth muscle cells and followed similar activation kinetics as p38MAPK phosphorylation (Fig. 3). Additionally, HSP27 activation was blocked in cells that were pretreated with SB203580. This evidence indicates that the activation of HSP27 by PDGF, IL-1β, and TGFβ in airway myocytes is due to activation of p38MAPK.

HSP27 is an actin binding protein (51) constitutively expressed at high levels in smooth muscle. In vitro, HSP27 is thought to function as an F-actin capping protein whose activity is regulated by phosphorylation by MAPKAP kinases-2/3 (44). Purified unphosphorylated mouse HSP25 inhibits actin polymerization but not phosphorylated HSP25 (52). Evidence for a role of HSP27 phosphorylation in the regulation of F-actin dynamics has been demonstrated in vivo in rodent fibroblasts. Lavoie et al. (45) showed that overexpression of wild type HSP27 increases the stability of F-actin filaments during incubation in the presence of cytochalasin D. They also demonstrated that phosphorylation of HSP27 is necessary for the modulation of actin remodeling by overexpression of a HSP27 phosphorylation mutant that showed a dominant negative effect. In Fig. 4, we showed that HSP27 phosphorylation is increased in smooth muscle cells that were treated with PDGF, IL-1β, and TGFβ. Moreover, pretreatment with SB203580 led to a reduction in HSP27 phosphorylation, again indicating that p38MAPK activation is involved in HSP27 phosphorylation in myocytes.

To further demonstrate the role of HSP27 phosphorylation in airway smooth muscle cell migration, we used an adenovirus vector to overexpress a HSP27 phosphorylation mutant (45). Cells that expressed the mutant HSP27 failed to migrate when treated with PDGF (Fig. 5). This is consistent with the hypothesis that HSP27 phosphorylation promotes F-actin remodeling, which is necessary for smooth muscle cell migration. In control experiments, cells that were uninfected or cells that were infected with an adenovirus vector lacking a cDNA insert migrated normally when treated with PDGF. 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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