A Specific Mechanomodulatory Role for p38 MAPK in Embryonic Joint Articular Surface Cell MEK-ERK Pathway Regulation*

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Mechanisms regulating cell behavior and extracellular matrix composition in response to mechanical stimuli remain unresolved. Our previous studies have established that the MEK-ERK cascade plays a specific role in the mechano-dependent joint formation process by promoting the assembly of pericellular matrices reliant upon hyaluronan (HA) for their integrity. Here we demonstrate: (i) novel cross-talk between p38 MAPK and MEK-ERK signaling pathways that is specific for mechanical stimuli and (ii) a role for p38 MAPK in facilitating HA production by cells derived from the articular surface of embryonic chick tibiotarsal joints. We find that p38 MAPK blockade restricts pericellular assembly of HA-rich matrices and reduces basal as well as mechanical strain-induced release of HA. p38 MAPK blockers potentiated early strain-induced increases but restricted sustained increases in MEK/ERK phosphorylation at later times; c-Fos hyperphosphorylation at threonine 325 was found to parallel this p38 MAPK-mediated modulation of ERK activation. In contrast, p38 MAPK inhibitors had no detectable effect on the ERK activation induced by fibroblast growth factor 2 or pervanadate, a phosphatase inhibitor, and MEK inhibitors did not influence p38 MAPK phosphorylation, confirming both the specificity and unidirectionality of p38 MAPK-ERK cross-talk. Immunoochemical and immunoblotting studies revealed constitutive p38 MAPK activation in cells at, or derived from, developing articular joint surfaces. Unlike the MEK-ERK pathway, however, p38 MAPK was not further stimulated by mechanical stimulation in vitro. Thus, p38 MAPK specifically facilitates ERK activation and downstream signaling in response to mechanical stimuli. These results suggest that constitutively active p38 MAPK serves an essential, permissive role in mechanically induced changes in ERK activation and in the accumulation of HA-rich extracellular matrices that serve a key role in joint development.

Mitogen-activated protein kinases (MAPKs)3 are ubiquitous serine/threonine kinases that are activated by diverse extracellular stimuli, including growth factors, cytokines, and physiological mechanical signals (1–3). MAPKs are essential for transducing signals from the cell surface that regulate diverse cellular behaviors, such as those coordinating development, proliferation, and differentiation. Much recent emphasis has been placed on members of three well characterized mammalian MAPK families, comprising extracellular signal-regulated kinases (ERKs), p38 MAPKs, and c-Jun N-terminal kinases (JNKs) (4–6). Until relatively recently it was considered that these families were preferentially activated by certain types of signal: the ERK family by growth factors and the p38 MAPK and JNK families by cellular stress. It is now becoming increasingly clear, however, that the various MAPK pathways can also be co-activated by a single stimulus. The precise interaction between members of these distinct MAPK families and how they cooperate to control cell behavior are, however, ill-defined.

Mechanical stimuli control cell behavior in a broad range of circumstances and can modify cell-matrix interactions, utilizing ERK-1/2 as a point of convergence (7). We have recently found that mechanical stimuli activate ERK, via MEK, in cells derived from chick joint articular surfaces (AS) and that MEK-ERK pathway activation selectively regulates their extracellular matrix elaboration (8). This role for MEK-ERK signaling provides evidence for its involvement in the mechano-dependent regulation of cell behavior. It remains to be determined, however, whether other MAPK pathways also regulate matrix assembly by AS cells.

Activation of p38 MAPK and JNK family members has been associated with cellular responses to a wide range of cellular stresses such as free radicals, UV light, or heat shock or pro-inflammatory cytokines (9, 10). Indeed, the production of many inflammatory mediators requires p38 MAPK-mediated regulation of post-transcriptional events, and as such p38 MAPK has been identified as a therapeutic target in a range of inflammatory disorders, including those of the joint (10, 11). In addition to the classic cellular stresses, chondrocyte p38 MAPK is also subject to activation by mechanical perturbation (12). These studies indicate that p38 MAPK pathways may mediate mechanotransduction and highlight the possibility that responses to mechanical stimuli may involve interactions between distinct MAPK family members. There is indeed substantial evidence for interaction between p38 MAPK and ERK. This includes demonstration of their direct association as well as regulatory cross-talk (13–15). Thus, it has been shown that ERK-mediated events can be regulated by active p38 MAPK and conversely that MEK-ERK pathway activation modifies p38 MAPK signaling (16–21). Together, these studies raise the possibility that p38 MAPK may regulate cell behavior through its modulation of ERK activation.

Our studies in AS cells have shown that mechanically induced ERK activation is both rapid and sustained and that these kinetic characteristics are allied to the formation and maintenance of pericellular matri-
ces (8, 22). A general mechanism through which ERK activation kinetics may be interpreted has recently been proposed; the immediate early gene product c-Fos has been shown to function as a downstream molecular sensor of ERK signal duration that allows cells to respond appropriately to extracellular signals (23, 24). Here we show that the duration and extent of MEK-ERK pathway activation is regulated by unidirectional cross-talk from active p38 MAPK, and the functional significance of this is emphasized by commensurate modulation of extracellular matrix assembly. This regulation of ERK signaling is evident in resting and is potentiated in mechanically stimulated cells, in which it is associated with c-Fos hyperphosphorylation. A selective function for p38 MAPK in modulating mechanically induced changes in the MEK-ERK pathway signaling is further accentuated by the complete failure of pharmacological blockers of p38 MAPK to modify the activation of MEK-ERK signaling induced by treatment with a range of alternative stimuli, including growth factors. Our findings have implications for the development and use of p38 MAPK inhibitors as potential anti-inflammatory therapeutics.

**EXPERIMENTAL PROCEDURES**

**Immunolabeling**—Longitudinal 10-μm cryostat sections of day 13 embryonic chick metatarsal-phalangeal joints were immunolabeled with an antibody recognizing total (active and inactive) p38 MAPK (Santa Cruz Biotechnology) or phosphorylated p38 MAPK (New England Biolabs). After washing, sections were incubated with the appropriate fluorescein isothiocyanate-conjugated secondary antibody in Tris-buffered saline/0.01% Tween 20 (TBS-T) containing 20% chick serum (25). Immunofluorescence was monitored using a Zeiss confocal microscope as described previously (26).

**Isolation of Articular Surface Cells and Mechanical Strain Application**—AS cells were extracted from embryonic stage 42 (18 day) chick tibiotarsi by collagenase digestion (25). Primary cells, seeded onto plastic culture strips (Nunc), were grown to confluency in Dulbecco’s modified Eagle’s medium containing 5% chick serum and 50 μg ml⁻¹ ascorbic acid, and subsequently cultured for 18 h in Dulbecco’s modified Eagle’s medium without serum. Fresh medium was then added with or without SB203580, SB202190, or PD98059 (Calbiochem), or U0126 (Promega), for 1 h prior to the application of mechanical strain as described (27).

**ERK Activity Assay**—The erythrocyte exclusion assay (30) was assessed using the p38 MAPK inhibitor, SB203580. Briefly, cells were seeded (1 × 10⁵ cells) onto plastic strips (Nunc) onto which the cells had been seeded as previously described (25, 27–29). This essentially imparts tensile strain in a controlled manner by bending of the cell culture-treated plastic strips onto which cells have been previously seeded. Cells were incubated for the times specified, and control cells were unperturbed (static) but otherwise treated identically. Primary rat long bone osteoblasts were isolated as described previously and treated as described (27).

**Erythrocyte Exclusion Assay**—The erythrocyte exclusion assay, a measure of pericellular matrix assembly in vitro, was performed as described (30). Briefly, cells were seeded (1 × 10⁵ cells) onto plastic strips and cultured with or without SB203580 (10 μM) for 24 h. Cells were subsequently fixed in 4% formalin and treated with an 80-μl volume of suspected sheep erythrocytes (Sigma, 1 × 10⁷ cells/ml) in phosphate-buffered saline within a defined 1.8-cm² area of tissue culture strip. Erythrocytes were allowed to settle for 10 min before exclusion by pericellular matrix was viewed using a Zeiss Axiosvert 100M inverted microscope, and images were collected using associated software (Zeiss LSM). Hyaluronan (HA)-dependent matrix assembly was verified by treatment for 1 h with 6 units ml⁻¹ Streptomyces hyalurolyticus hyaluronidase (Sigma).

**HA Assay**—An enzyme-linked immunosorbance-based assay was used to measure concentrations of HA released into the culture media 24 h after mechanical strain application (31). The assay is based upon the competition between HA absorbed on to the plate and HA free in solution for binding to biotinylated cartilage proteoglycan binding region (G1 domain) (32).

**Western Blotting**—ERK activation was examined in cell lysates collected 20 min, 1 h, 6 h, and 24 h post-strain. Protein content of lysates was measured using the bichinchoninic (BCA) assay following the manufacturer’s instructions (Pierce). Proteins in cell lysates (40 μg) were separated by SDS-PAGE (10%) and transferred to nitrocellulose (Hybond ECL). Membranes were incubated with the appropriate primary and horseradish peroxidase-conjugated secondary antibodies as previously described (17). Immuno-reactive bands were visualized by enhanced chemiluminescence (ECL, Amersham Biosciences) (33). Membranes were stripped and re-probed sequentially with each of the following antibodies as required (p-ERK1/2 and p-MEK1/2, New England Biolabs; ERK2, Transduction laboratories; p-p38 MAPK New England Biolabs; p38 MAPK Santa Cruz Biotechnology; p-JNK, Promega; and phospho-c-Fos Thr³²⁵ was a kind gift of Leon Murphy and John Blenis, Boston, MA) (23).

**p38 MAPK Immunoprecipitation and Kinase Assay**—p38 MAPK activation in cells exposed to mechanical strain in the presence or absence of p38 MAPK inhibitors (SB203580) was assessed using the p38 MAPK kinase assay kit (New England Biolabs) with minor modification to the manufacturer’s instructions to facilitate p38 MAPK blockade. Preliminary studies and the work of others (34) have revealed that inhibition of p38 MAPK phosphorylation required the presence of inhibitors during the kinase reaction. Therefore, p38 MAPK inhibitors were re-added during the kinase reaction for 30 min at 37 °C prior to the addition of ATP and ATF2 fusion protein.

**Densitometry and Statistical Analysis**—Immunoblot band intensity was evaluated by densitometric analysis (mean ± S.E.) and expressed as a fold change in experimental relative to control groups. Statistical significance was determined using the paired or unpaired Student’s t test as appropriate (Sigma Plot, version 8.0), and p values < 0.05 were considered statistically significant as detailed in the figure legends.

**RESULTS**

**p38 MAPK Is Activated at the Joint Line in Developing Limbs and Regulates Pericellular Coats in Vivo**—We have previously shown that ERK1/2 activation regulates behavior of articular joint line cells (35, 8). To determine whether p38 MAPK activation may contribute to regulating cellular behavior at the joint line, we examined the expression of total and active p38 MAPK in developing chick limbs (Fig. 1A). This showed that total (inactive plus active) p38 MAPK was predominantly expressed in cells of the fibrocartilaginous articular surface (AS) of joint elements, which also showed active p38 MAPK expression. Chondrocytes in epiphyses and bordering vascular canals also expressed lower levels of total and active p38 MAPK (Fig. 1A). Higher magnification showed cytoplasmic localization of p38 MAPK (data not shown).

A central feature of AS cell behavior is their ERK1/2-dependent formation of HA-rich pericellular matrices (8). To determine whether activation of the p38 MAPK pathway contributes to this aspect of AS cell function, we evaluated the effect of p38 MAPK inhibitors on HA-dependent pericellular coats. Addition of the p38 MAPK inhibitor, SB203580, to AS cells for 1 h and assessment of pericellular coats by erythrocyte exclusion assay 24 h later, revealed a marked reduction in...
coat size (Fig. 1B). These results suggest that activation of p38 MAPK positively contributes to the prevalence of pericellular coats and therefore to AS cell differentiation.

**Mechanical Strain Activates ERK but Not p38 MAPK**—Further support for a role for p38 MAPK in controlling AS cell differentiation was also found by measuring basal and mechanical strain-induced HA release. As shown previously, application of mechanical strain enhanced HA release (25), and here we found that both basal and strain-induced HA release was significantly reduced by treatment with SB203580 (Fig. 1C). This emphasizes a role for p38 MAPK in the response of AS cells to mechanical strain. To investigate the role of p38 MAPK activation and its relationship to ERK1/2 activation, AS cells were exposed to mechanical strain, and p38 MAPK and ERK1/2 activation was assessed by immunoblotting with phospho-specific antisera. Equal protein loading was confirmed in these experiments by measuring total ERK and total p38 MAPK. Consistent with previous studies, we found that ERK1/2 activation in AS cells was enhanced by strain (8, 35). In marked contrast, we also found that, although a high level of constitutive p38 MAPK activation was exhibited by AS cells, no further activation was induced by the application of mechanical strain stimuli (Fig. 2A). Similarly, probing for active-JNK also failed to disclose any activation by strain (data not shown). As shown in Fig. 2B, strain-induced ERK1/2 activation was sustained for up to 24 h after strain. Densitometric analysis (Fig. 2B) showed a significant 6-fold increase in ERK1/2 activation 20 min after strain; less marked increases at 1–24 h reflected a time-dependent rise in basal ERK1/2 activation. On occasion, basal increases partly masked strain-related increases in ERK1/2 activation at later times.

**Biphasic Modulation of ERK Activation by p38 MAPK**—Previous studies had highlighted evidence of cross-talk between p38 MAPK and ERK1/2 (15, 17, 18–20, 36, 37). In an attempt to identify a role for p38 MAPK we were, therefore, prompted to investigate the effect of p38 MAPK blockade on basal and strain-induced ERK1/2 activation. We
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Inhibition of p38 MAPK Also Modulates ERK Activation in Rat Osteoblasts—To determine whether p38 MAPK exerts a similar influence in other skeletal cells or whether this effect was species specific, we examined whether inhibition of the p38 MAPK pathway similarly influenced ERK1/2 activation in rat long bone-derived osteoblasts exposed to identical mechanical strain stimulus (40, 41). In common with AS cells, osteoblasts cultured in the presence of SB202190 showed enhanced strain-induced ERK1/2 activation at 20 min and a restriction after 24 h (Fig. 5). Similar effects were induced following treatment with SB203580 (data not shown), and, as previously observed in AS cells, osteoblast MEK1/2 activation patterns were similar to ERK1/2 (Fig. 5). Together these findings indicate that p38 MAPK-ERK1/2 cross-talk is not restricted to avian cells and is also evident in cells derived from other skeletal tissues.

MEK1/2 Inhibitors Do Not Affect p38 MAPK Activation—Inhibition of ERK1/2 in other cell types is known to influence p38 MAPK (15). To address the possibility that cross-talk between p38 MAPK and ERK1/2...
Representative blots from two separate experiments are shown. p-MEK. Total ERK blotting was included to demonstrate equal protein loading. Representative blots from three experiments are shown.

FIGURE 5. p38 MAPK inhibitors modify strain-induced ERK activation in primary rat osteoblasts. Rat long bone-derived osteoblasts were serum-deprived and exposed to SB202190 (10 μM) prior to the application of mechanical strain. Cells were cultured for the times indicated, and lysates were subjected to immunoblotting to detect p-ERK and p-MEK. Total ERK blotting was included to demonstrate equal protein loading. Representative blots from two separate experiments are shown.

FIGURE 6. MEK inhibitors do not modify basal, strain-, or pervanadate-induced activation of p38 MAPK. A, chick AS cells were serum-deprived and exposed to U0126 (10 μM) prior to application of mechanical strain. Cells were cultured for the times indicated, and lysates were subjected to Western analysis to detect p-ERK, total ERK, p-p38 MAPK, and total p38 MAPK. B, chick AS cells were serum-deprived and treated with sodium pervanadate (1–100 μM) for the times indicated. Lysates were assayed for ATF2 activity (p-ATF2) using a p38 MAPK kinase assay according to the manufacturer’s instructions. Representative blots from two separate experiments are shown.

is bidirectional, ERK1/2 and p38 MAPK phosphorylation was examined in AS cells pre-treated with MEK1/2 inhibitors (42). Inhibition of the MEK-ERK pathway by either 10 μM U0126 (Fig. 6A) or PD98059 (data not shown) had no discernable effect on p38 MAPK phosphorylation. To investigate whether alternative routes of p38 activation are similarly unaffected by MEK1/2 inhibition, AS cells were treated with sodium pervanadate, a phosphatase inhibitor, and AT2F2 phosphorylation was assessed. This showed that U0126 treatment also failed to modify pervanadate-induced p38 MAPK activity (Fig. 6B), demonstrating the cross-talk from p38 MAPK to ERK1/2 was unidirectional in control and mechanically strained AS cells.

Agonist-induced ERK Activation Is Not Modulated by p38 MAPK—It is possible that p38 MAPK-ERK cross-talk persistently modulates ERK activation status in AS cells irrespective of the applied stimulus. To examine the possibility that p38 MAPK-ERK cross-talk is a ubiquitous characteristic of MEK-ERK pathway regulation in AS cells, or whether it is a feature of signaling that is selectively associated with the response to a mechanical stimulus, AS cells were exposed to FGF2 (0.02–2 ng/ml for 10–180 min) in the absence or presence of SB203580. ERK1/2, but not p38 MAPK, exhibited dose-dependent FGF2-induced activation, which was maximal after 10 min. However, we found that, in contrast to its effects on mechanical strain-induced ERK1/2 activation (Fig. 3), SB203580 failed to exert any modifying effect on FGF2-induced ERK1/2 activation (Fig. 7A). Similar results were also observed with both epidermal growth factor (data not shown) and sodium pervanadate (Fig. 7B), indicating that p38 MAPK-ERK1/2 cross-talk appears to be dependent upon the specific stimulus for ERK1/2 activation. Thus, use of p38 MAPK blockers discloses cross-talk in circumstances where mechanical strain, but not FGF2, epidermal growth factor, or pervanadate, is the stimulus for MEK-ERK pathway activation.

Inhibition of p38 MAPK Modifies ERK and c-Fos Activation in Parallel—c-Fos has recently been described as a sensor for ERK signal duration (23). ERK1/2 phosphorylates c-Fos at Ser327 and causes hyperphosphorylation at Thr325 and Thr321. Thus, Thr325 phosphorylation requires sustained ERK1/2 activation (23). To determine whether Thr325 phosphorylation of c-Fos correlates with the duration of ERK activation, we examined the effect of SB203580 on strain-induced c-Fos phosphorylation using a phospho-specific Thr325 antibody (p-c-Fos Thr325). We found that, in parallel with ERK1/2, p-c-Fos Thr325 expression in SB203580-treated cells was indeed enhanced at early and restricted at later times after strain application (Fig. 8). Together these results suggest that constitutively active p38 influences the duration and extent of ERK1/2 activation and hyperphosphorylation of one of its downstream targets c-Fos.

DISCUSSION

Our previous studies have established that the MEK-ERK cascade plays a specific role in joint formation by promoting the assembly of pericellular matrices that depend upon HA for their integrity (8). Here we show: (i) that p38 MAPK blockade restricts HA release and pericellular matrix assembly, (ii) that p38 MAPK influences mechanically induced ERK activation but does not contribute to growth factor-induced phosphorylation in primary articular surface cells; (iii) that this control of mechanomodulatory MEK-ERK signaling by p38 MAPK is biphasic and unidirectional; (iv) that p38 MAPK is constitutively active in cells at, or derived from, developing articular joint surfaces, and (v) that, unlike the MEK-ERK pathway, p38 MAPK is not stimulated further by mechanical stimuli in vitro. These novel findings indicate that active p38 MAPK modulates ERK signaling and HA-rich pericellular matrix assembly, suggesting a key role in developing joints (Fig. 9).

There is substantial evidence for cross-talk between various MAPK pathways. Unidirectional cross-talk from MEK-ERK to p38 MAPK pathways has for example been demonstrated in HeLa cells, primary cardiomyocytes, and perfused rat jejunum (15, 43, 44). Our studies showing that phosphorylation and activity of p38 MAPK are unaffected by the MEK inhibitors, U0126 or PD98059, indicate that the MEK-ERK pathway does not modulate p38 MAPK activation in primary AS cells. More extensive data support a complementary, reciprocal control of MEK-ERK signaling by p38 MAPK. This includes p38 MAPK-dependent activation of ERK in HEK293 and PC12 cell lines (36, 45) as well as p38 MAPK-dependent suppression of ERK in hepatoma, myoblast, and leukemia cell lines in vitro and in a remnant kidney model in vivo (20, 21, 37, 46–49). We have also previously shown suppression of MEK-ERK signaling by p38 MAPK in primary human endothelial cells (17). Our current findings show that p38 MAPK is also a pivotal regulator of ERK activation in primary cells derived from developing joint articular surfaces. Moreover, we show that p38 MAPK-ERK cross-talk in these cells is stimulus-dependent, being uniquely restricted to regulation of mechanically induced MEK-ERK signaling and not that induced by other ERK activators, including growth factors or alternative modes of

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promoting an accumulation of active ERK, such as pervanadate. A proposed model for the relationship between p38 MAPK and ERK activation as well as their cross-talk in response to mechanical stimuli is illustrated in Fig. 9.

Interactions between signaling pathways are thought to be regulated by interconnections, either at junctions or nodes, i.e. junctions integrating signals from multiple sources and nodes transmitting signals to multiple downstream targets (50). A single signaling component can function as one or both of these interconnectors. Our studies show that MEK and ERK phosphorylation are coordinately regulated by p38 MAPK. This, together with the unidirectional nature of the cross-talk, suggests that p38 MAPK targets the MEK-ERK cascade at, or upstream of MEK. Targeting of the MEK-ERK cascade by p38 MAPK in the context of mechanical modulation, but not in response to growth factors, indicates that MEK-ERK signaling and downstream events, such as c-Fos phosphorylation, are coordinately modified by p38 MAPK blockage. Our studies in AS cells and osteoblasts indicate differential usage by strain or growth factors of signaling elements upstream of MEK-ERK. Although these elements remain to be identified, it has been shown that active p38 MAPK can complex with ERK to prevent its phosphorylation by MEK-1/2 (13) and that mechanical shear stress promotes formation of signaling complexes in endothelial cells (51). The possibility that p38 MAPK-mediated modulation of ERK in skeletal cells occurs via signal complex formation or enzyme association induced selectively by mechanical stimuli is under examination.

Many stimuli, including mechanical perturbation, use ERK as a point of convergence, and it has been proposed that ERK discriminates between signals on the basis of the strength and duration of activation (1–3, 52). Consistent with our previous studies (8) we show that mechanical strain provokes rapid and sustained increases in ERK activation and that this is achieved in the absence of any modification in phospho-p38 MAPK expression. We found that p38 MAPK blockers potentiated the early strain-induced increases in ERK activation but restricted sustained increases at later times. This suggests that p38 MAPK serves dual roles: dampening early but promoting late onset strain-induced ERK activity. This is consistent with a capacity for p38 MAPK to both positively and negatively regulate ERK activation. In the context of joint formation it is clear that the high levels of active p38 MAPK disclosed at the joint line are likely, therefore, to facilitate local mechanically induced ERK activation. The significance of p38 MAPK in matrix assembly is also endorsed by the ability of SB203580 to diminish coat formation and HA release. Our findings indicate that p38 MAPK blockade does, however, also regulate ERK activation, albeit to a lesser extent, in non-strained AS cells in vitro. This supports usage of similar signaling modalities in resting and strain-stimulated but not growth factor-treated AS cells.

It remains possible that our findings utilizing pharmacological blockade of p38 MAPK represent some nonspecific influence of these inhibitors. However, several of our findings are inconsistent with such lack of specificity. First, our studies using SB203580 and SB202190 produced equivalent effects on p38 MAPK activity and c-Fos phosphorylation. Secondly, these effects exhibited dose dependencies consistent with selective blockade of p38 MAPK (53). Lastly, similar mechanomodulatory influences of p38 MAPK inhibition were evident in another skeletal cell type, namely osteoblasts. Fourthly, p38 MAPK blockade by either inhibitor produced concomitant effects on MEK and ERK phos-
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Phosphorylation of ERK can be modulated both by upstream kinases and by phosphatases (PPases) (54, 55). Modulation of PPase activity could, therefore, contribute to regulating ERK activation in AS cells. Indeed, our findings indicate that a broad specificity PPase inhibitor (pervanadate) strongly promoted ERK phosphorylation but that, unlike mechanical strain-induced ERK activation, this was unaffected by blockade of p38 MAPK. Thus, MEK-ERK activation in response to strain is unlikely to rely solely on PPase activity. It is also unlikely, therefore, that p38 MAPK achieves its regulation of ERK via modification of PPases. Moreover, support for a mechanism acting independently of specific MAPK-phosphatases is provided by our finding that MEK phosphorylation parallels ERK in mechanically strained cells treated with p38 MAPK inhibitors. Because MAPK-phosphatases act specifically upon the dually phosphorylated MAPKs, such as ERK, it would have been expected that a mechanism reliant upon changes in MAPK-phosphatase activity should target ERK without modifying MEK phosphorylation. Thus, neither strain nor p38 MAPK appear to utilize either constitutive or induced phosphatases as a principal mechanism for regulating ERK in cells derived from articular surfaces.

Consistent with previous findings (8), we show that a transient mechanical strain stimulus results in both acute and sustained MEK-ERK activation. Recent studies demonstrated that c-Fos hyperphosphorylation at threonine \(^{325}\) is one molecular sensor of the duration and strength of ERK activity (23, 56). We show that this mode of interpreting ERK activation is evident in primary AS cells and, importantly, that p38 MAPK influences c-Fos hyperphosphorylation either directly or via p38 MAPK-ERK cross-talk. In addition to our demonstration that ERK regulates pericellular matrix assembly (8), we now show that basal and strain-induced HA release and pericellular coat formation are also dependent upon p38 MAPK.

Molecular mechanisms responsible for transducing mechanical stimuli into changes in cell behavior remain unresolved in many tissues. A central role for ERK in the precise regulation of normal joint development, which requires mechanical input (57–61) and involves a local accumulation of HA-rich extracellular matrix, is, however, established (25, 8, 62, 63). Our examination of p38 MAPK distribution in developing limbs revealed the presence of cells expressing constitutively active p38 MAPK at sites of joint cavity formation and is endorsed by our demonstration that cultured cells derived from these sites also conserve p38 MAPK in the active state. Increasing emphasis on use of p38 MAPK inhibitors as anti-inflammatory therapies for joint disorders may therefore require re-evaluation (38). Our findings show that p38 MAPK regulates mechanically induced changes in extracellular matrix formation and, therefore, have implications for use of p38 MAPK-targeted anti-inflammatory drugs. It is apparent that constitutively active p38 MAPK serves an essential, selective, and permissive role in mediating mechanically induced changes in ERK activation and contributes to the accumulation of the HA-rich extracellular matrix.

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