Members of the epidermal growth factor (EGF) family of ligands and their receptors regulate migration and growth of intestinal epithelial cells. However, our understanding of the signal transduction pathways determining these responses is incomplete. In this study we tested the hypothesis that p38 is required for EGF-stimulated intestinal epithelial monolayer restitution. EGF-stimulated migration in a wound closure model required continuous presence of ligand for several hours for maximal response, suggesting a requirement for sustained signal transduction pathway activation. In this regard, prolonged exposure of cells to EGF activated p38 for up to 5 h. Furthermore genetic or pharmacological blockade of p38 signaling inhibited the ability of EGF to accelerate wound closure. Interestingly p38 inhibition was associated with increased EGF-stimulated ERK1/ERK2 phosphorylation and cell proliferation, suggesting that p38 regulates the balance of proliferation/migration signaling in response to EGF receptor activity. Activation of p38 in intestinal epithelial cells through EGF receptor was abolished by blockade of Src family tyrosine kinase signaling but not inhibition of phosphatidylinositol 3-kinase or protein kinase C. Taken together, these data suggest that Src family kinase-dependent p38 activation is a key component of a signaling switch routing EGF-stimulated responses to epithelial cell migration/restitution rather than proliferation during wound closure.

Appropriate regulation of cell migration is critical for maintenance of a healthy intestinal epithelial lining in at least two ways. The continuously renewing monolayer of cells covering the gastrointestinal tract undergoes complete turnover every few days in a process that involves the movement of newly generated cells from the proliferative compartment in the lower crypts to the small intestinal villi or colonic surface epithelia (1, 2); thus, orderly cell migration contributes to proper morphology and function of the gastrointestinal tract in the disease-free state. Furthermore the ability of epithelial cells to migrate and close a wound in the mucosal lining allows for restitution of the epithelium much more rapidly than by enhanced proliferation and is thus an early response for maintenance of barrier and absorptive functions in the face of environmental or inflammatory damage. Increased cell migration has been observed in experimentally injured epithelia (3–5) as well as in disease states such as intestinal mucosal ulceration (6–8) and inflammatory conditions (9–11). While the importance of cell motility in the dynamic maintenance of the gastrointestinal tract is clear, the molecular mechanisms regulating intestinal cell migration are not yet fully understood.

One regulatory mechanism influencing intestinal epithelial cell migration is signaling initiated by soluble growth factors, including epidermal growth factor (EGF). 3 EGF is the canonical member of a family of peptide growth factors (also including betacellulin, heparin-binding EGF-like growth factor, amphiregulin, transforming growth factor-α, and epiregulin) that act as ligands for the EGF receptor (EGFR, also known as ErbB-1) (12). EGFR is a 170-kDa type I transmembrane glycoprotein containing a ligand-binding ectodomain, a single hydrophobic transmembrane region, and a cytoplasmic tail that includes a tyrosine kinase domain and docking sites for a variety of signaling effectors (13, 14). The receptor is widely expressed in mammalian epithelial tissues, and binding of ligands impacts a variety of cell physiology parameters such as cell growth (15–17), survival or apoptosis (18–22), secretion and ion transport (23–25), and oncogenic transformation (13, 26–28). In addition, reports from this and other laboratories clearly implicate EGFR signaling as a potent inducer of intestinal cell migration and wound healing (10, 29, 30). EGF application to cultured mouse intestinal epithelial cells dramatically increases migration into a wounded area (31), and similar results have been reported with rabbit duodenal organ cultures (32) as well as cultured human colonic cell lines or epithelia (33–35). Furthermore a recent clinical study indicates that topical EGF application is effective in treatment of ulcerative colitis (36), suggesting that the promotion of wound healing by this factor in vitro effectively models in vivo responses.

EGF ligand binding stimulates receptor homodimerization as well as heterodimerization with other ErbB family receptors (37) and activation of a number of target proteins regulating cytoskeletal rearrangement and cellular migration (38, 39). Several parallel pathways downstream of the EGFR, including phospholipase C-γ and phosphatidylinositol (PI) 3-kinase/Akt, appear to be required for EGF stimulation of cell migration in intestinal epithelial cells (31).

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¶ The abbreviations used are: EGF, epidermal growth factor; EGFR, EGF receptor; MAPK, mitogen-activated protein kinase; PI, phosphatidylinositol; YAMC, young adult mouse colon; ERK, extracellular signal-regulated kinase; JNK, c-Jun NH2-terminal kinase; SAPK, stress-activated protein kinase; PKC, protein kinase C; HGF, hepatocyte growth factor; PBS, phosphate-buffered saline; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase.
Another downstream signaling module responsive to EGF in some cell systems is the p38 kinase (19, 40, 41). A member of the MAPK signaling family, p38 is largely associated with cellular stress responses and apoptosis (42–44). It is required for tumor necrosis factor-induced apoptosis in intestinal cell models (45, 46) or for ErbB-2-dependent apoptosis in breast carcinoma cells overexpressing the EGFR (19). Interestingly, p38 activation has been noted in an in vitro intestinal epithelial restitution model (47) and in wounded corneal epithelial tissue (48), suggesting that under some circumstances p38 may be involved in regulating cell motility. However, a formal requirement for p38 MAPK in intestinal epithelial cell migration has not been established, and the role of this kinase in growth factor-stimulated migration is also unknown.

The purpose of this study was to further characterize the mechanism of EGF-stimulated intestinal epithelial cell migration. Using our laboratory’s in vitro cell restitution model system, we determined that EGF-stimulated migration requires prolonged presence of ligand concomitant with activation of p38 MAPK. Use of pharmacological inhibitors and dominant-negative constructs showed that Src family kinase-dependent activation of p38 is a key component of EGF-induced intestinal epithelial cell migration/wound closure but is not required for basal migration. Furthermore our data demonstrated that p38 is a molecular proliferation/migration “switch” capable of determining the outcome of EGFR signaling in intestinal epithelial cells.

MATERIALS AND METHODS

Cell Culture—The conditionally immortalized young adult mouse colon (YAMC) cell line has been previously described and was established from the colonic epithelium of H-2Kd-tsA58 (Immorte) mice (49). These cells express a heat-labile SV40 large T antigen expressed under the control of an interferon-γ-inducible promoter. EGFR-null (EGFR−/−) mouse colon epithelial cells were established in a similar fashion from EGFR-null heterozygous mice crossed with the Immortomouse (49, 50). Cells were maintained on rat tail collagen (Mediatech, Herndon, VA)-coated plates in RPMI 1640 medium with 5% fetal bovine serum, 5 units/ml mouse interferon-γ (Intergen, N愿cos, GA), 100 units/ml penicillin and streptomycin, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml apro tinin, and 1 mM phenylmethylsulfonyl fluoride. 500 μg of each lysate was incubated for 1 h at 4 °C with 1 μg of anti-Src, anti-Lck, or anti-Lyn antibody and then for 1 h with Protein A/G PLUS-agarose beads (Santa Cruz Biotechnology). Beads were collected by centrifugation, washed three times with lysis buffer, and boiled in Laemmli sample buffer for SDS-PAGE and Western blot analysis.

Proliferation Assays—YAMC cells were plated in 96-well dishes (5 × 103 cells/well) and maintained under nonpermissive conditions for 16 h before the beginning of treatments. Some cultures were exposed to growth factors and/or signaling inhibitors for 24 h. Cells were counted at 0 and 24 h using a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphen-2-(4-sulfophenyl)-2H-tetrazolium-based colorimetric proliferation assay kit (Promega Corp.). Reported values reflect averages of at least 12 replicate wells.

DNA Constructs and Transfections—A dominant-negative p38 construct (53) was the gift of Dr. Melanie Cobb (Southwestern University, Dallas, TX). A dominant-negative Src construct (Y527F/K295R) was the gift of Dr. Steve Hanks (Vanderbilt University, Nashville, TN). For transfections, cells were seeded at low density (~25% confluence) in 35-mm dishes. After 24 h of culture, the medium was replaced under permissive conditions but with no antibiotics, and cells were grown to 90% confluence. Transfections were then performed using the Lipo-fectAMINE 2000 (Invitrogen) transfection reagent using 1 μg of DNA/cell and following the manufacturer’s recommendations. 24 h after transfection, cells were shifted to nonpermissive conditions for 16 h before use in an experiment.

Statistical Analysis—In all experiments assessing the statistical significance of differences between mean values was assessed with paired Student’s t test analysis. The minimum level of statistical significance was set at 0.05. Unless otherwise stated, all data are representative of at least three independent experiments.

RESULTS

EGF-stimulated Intestinal Epithelial Cell Migration Requires Sustained Presence of Ligand—Previous reports from this laboratory have demonstrated that EGF exposure stimulates intestinal epithelial cell migration in an in vitro wound closure model (31). This response is dependent upon activity of the phospholipase C-γ/PKC and PI 3-kinase/Akt signaling cascades. Recent evidence also implicates Src inhibitor-sensitive

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Statistical Analysis—In all experiments assessing the statistical significance of differences between mean values was assessed with paired Student’s t test analysis. The minimum level of statistical significance was set at 0.05. Unless otherwise stated, all data are representative of at least three independent experiments.
signaling in extracellular signal-induced migration of these cells (52). One question that has not been addressed, however, is whether EGF-stimulated migration is regulated at the receptor level by a transient signal that is rapidly translated to intracellular second messengers, thus dispensing with the requirement for further ligand presence, or whether sustained EGFR occupancy and activation are required. Other cell physiological programs, such as the MAPK-dependent switch between growth and differentiation of PC12 pheochromocytoma cells, are determined by a combination of signal strength and signal duration (54). Similarly many EGF-stimulated signaling cascades are activated in a transient fashion by receptor ligation, but it is possible that sustained signaling is also required. For example, EGF-stimulated proliferation requires continuous ligand presence in some systems (16, 55).

To further investigate the dynamics of signaling cascades involved in EGF-stimulated intestinal epithelial cell migration, we asked whether transient EGFR ligation is sufficient to enhance cellular migration or whether this process requires sustained presence of the EGF ligand. YAMC cell wounds were prepared as described under “Materials and Methods,” and some were treated with EGF (10 ng/ml) either continuously or for various periods of time followed by removal of EGF and replacement with EGF-free medium. Continuous EGF exposure stimulated wounds to close at ∼150% of control rates in an 8-h migration experiment (Fig. 1A). In contrast, brief EGF exposure did not stimulate cell migration beyond basal levels. Interestingly pulse treatment with EGF for various times to determine the minimum duration of receptor occupancy required for maximal restitution (Fig. 1B) showed that after 4 h the presence of ligand is dispensable for full migration.

Taken together with our previous finding that intestinal epithelial cell migration is sensitive to EGFR tyrosine kinase inhibitors (31), these data indicate that sustained receptor-induced signal transduction is required for EGF-accelerated restitution. One alternate, albeit unlikely, possibility that must be formally ruled out is that the promigratory effects of sustained EGF exposure are actually a result of ligand-induced EGFR down-regulation. To determine whether loss of EGFR enhances intestinal cell migration, YAMC cell cultures were incubated with EGF for 16 h to deplete their EGFR pool, washed, given medium without EGF, and then compared with control cells in a migration assay (Fig. 1C). Basal migration of cells depleted of EGFR was not faster than that in cells expressing normal levels of receptor; in fact, down-regulation of the EGFR impaired basal migration somewhat, suggesting that at least part of the basal migration response is due to EGFR activity.

*p38 MAPK, ERK1/ERK2, and JNK/SAPK Are Activated in a Sustained Fashion by EGF in YAMC Cells—*EGF-stimulated cell migration requires the sustained presence of ligand; therefore, we sought to identify downstream signaling pathways with similar kinetics. To determine the duration of the signal transduction pathway activation by sustained EGFR signaling in YAMC cells, cultures were treated with 10 ng/ml EGF for various periods of time, lysed, and subjected to Western blot analysis using antibodies directed against the active phosphoforms of p38 MAPK, ERK1/ERK2 MAPK, Akt, and JNK/SAPK. All four of these molecules were rapidly activated by EGF (Fig. 2). While reduced levels of phospho-p38, -ERK, and -JNK/SAPK were detected after 5 h of EGF exposure, their activity was still substantially elevated compared with controls at this time. In contrast, Akt activation was detected only acutely following EGF treatment of YAMC cells.

*p38 Inhibition Blocks EGF-stimulated Migration but Potentiates EGF-stimulated Proliferation in YAMC Cells—*p38 MAPK, ERK1/2, and JNK/SAPK are all activated by EGF in a sustained fashion in YAMC cells. Furthermore previous studies have shown that p38 and ERK are active in intestinal epithelial cells migrating to close a wound (47). However, MEK and JNK inhibitors do not block EGF-stimulated YAMC cell motility (Ref. 56 and results below). Therefore, we tested whether p38 activity is necessary for EGF-induced cell migration. YAMC cell cultures were pretreated with the p38 inhibitor SB202190 (10 μM) for 1 h and tested in our wound healing assay with or without EGF. To determine whether the observed
effects were specific for EGF-stimulated pathways as opposed to generalized modulation of any signal for cell motility, SB202190 was also tested against HGF/scatter factor, which stimulates cell motility through different mechanisms than EGF (57). SB202190 pretreatment had little effect on basal or HGF-stimulated YAMC cell migration but efficiently abrogated EGF-accelerated migration (Fig. 3A). In contrast, the MEK inhibitor U0126 had little effect on migration in this assay. Fig. 3B shows inhibition of EGF-stimulated p38 and ERK1/ERK2 phosphorylation by SB202190 and U0126, respectively. Blockade of EGF-induced migration was also observed in another intestinal epithelial cell line, the IEC-18 rat ileal crypt line (Fig. 3C). Similar results were seen with another p38 inhibitor, SB203580 (data not shown), and with YAMC cells transiently transfected with a dominant-negative p38 (Fig. 3D). Thus, p38 activity is required for EGF-stimulated cell migration but not basal migration or migration stimulated by unrelated growth factors. In this regard, a previous report showed p38 activation in IEC-6 rat intestinal epithelial cells during wound restitution (47). To determine whether p38 is also activated by wounding alone in colon epithelial cells, we prepared wounds in monolayers of YAMC or EGFR−/− mouse colonic epithelial cells using an electrophoresis comb to make concentric rings of migrating cells. Cell lysates of control, EGF-treated, or wounded cells were analyzed by Western blot analysis for p38 activation.

**Fig. 2.** EGF stimulates Akt, ERK, JNK, and p38 activation in YAMC cells. YAMC cultures were exposed to EGF for 15 min (15') or 5 h. Whole cell lysates were prepared and subjected to Western blot analysis using antibodies directed against the fully phosphorylated (active) forms of Akt, ERK1/ERK2, JNK/SAPK, and p38. Total p38 blotting is shown as a loading control. \( p - \), phospho-.

**Fig. 3.** p38 inhibition blocks EGF-stimulated intestinal epithelial cell migration. A, confluent YAMC monolayers were preincubated with the p38 inhibitor SB202190 (10 \( \mu \)M) (i) or the MEK inhibitor U0126 (10 \( \mu \)M) (ii) for 1 h before being subjected to a wound healing assay using either EGF (10 ng/ml) or HGF (10 ng/ml). Inhibitors were present for the duration of the experiment. Wound closure rates relative to control at 8 h are shown. \( \ast \), \( p < 0.0001 \) versus control; \( \ast \ast \), \( p < 0.0001 \) versus EGF alone. B, YAMC cell cultures were pretreated with SB202190 or U0126 before exposure to 10 ng/ml EGF for 5 min (5'), and whole cell lysates were analyzed by Western blot for p38 (i) or ERK1/ERK2 (ii) activation as in Fig. 2. C, confluent IEC-18 monolayers were preincubated with SB202190 before being subjected to a wound healing assay using EGF. D, YAMC cells were transiently transfected with vector alone or dominant-negative p38 as described under “Materials and Methods” and subjected to a wound healing assay with EGF (10 ng/ml). Con, control; Vec, vector; DN, dominant-negative; SB, SB202190; \( p - \), phospho-.
p38 MAPK Regulates EGF-induced Migration of Intestinal Cells

status. As expected, 15 min of EGF exposure stimulated p38 in the YAMC cells but not in EGFR-deficient colonocytes (Fig. 4). However, wounded monolayers of both cell lines displayed elevated phospho-p38 content.

During the epithelial wound healing process in vivo, rapid cell migration to cover denuded areas is followed by proliferation to replace lost cells (4, 7, 8, 58). These processes are temporally distinct, and while EGF can stimulate both migration and proliferation, cell context and signaling state may determine which response is the outcome to growth factor binding. Given that p38 is activated in wounded intestinal epithelial cells (Fig. 4) but required only for EGF-stimulated, not basal, migration (Fig. 3), we asked whether this MAPK might function as a state-sensing switch selecting between cell motility and proliferation in response to an EGFR signal. The effects of p38 inhibition on EGF-stimulated intestinal cell proliferation were also tested. YAMC cells were grown in 96-well plates and exposed to EGF for 24 h with or without SB202190 or U0126 pretreatment, and cell number was determined as described under “Materials and Methods.” Increased cell number over control (no EGF) populations is shown in Fig. 5A. In contrast to its effects on EGF-stimulated YAMC cell migration, blockade of p38 signaling potentiated the ability of EGF to stimulate cell proliferation in this model. p38 inhibition in the absence of EGF slightly decreased basal cell growth, but while EGF stimulated cell growth at roughly 150% over control in cells with active p38 (Me2SO-treated control), pretreatment with SB202190 allowed EGF to stimulate growth at 220% of the SB202190-only control during the 24-h experimental period. On the other hand, blockade of ERK1/ERK2 activation by MEK inhibited both basal and EGF-stimulated proliferation in this model. Interestingly p38 blockade also potentiated the activity of the mitogenic ERK1/ERK2 kinases after EGF exposure (Fig. 5B), although no effect on base-line ERK1/ERK2 activity was seen with p38 inhibitors in this system. Thus, p38 appears to function as a switch that determines whether the outcome of EGFR ligation is proliferation or migration in intestinal epithelial cells. Results similar to these (not shown) were observed in the IEC-18 cell model.

Late Phase p38 Activation in YAMC Cells Is Required for EGF-stimulated Cell Migration—Data from a variety of experimental systems (54, 59–61) indicate that both the strength and duration of MAPK signaling cascades are important in determining the outcome of signaling. To examine this issue in the context of p38 MAPK in intestinal cell migration, we treated YAMC cells with EGF for various times between 5 min and 5 h and analyzed p38 activation at these times by Western blotting using anti-phospho-p38 antibody. Activation of p38 by EGF in these cells was biphasic (Fig. 6A) with an early peak at 5–15 min followed by a decrease from 30–60 min and a later phase of less intense but sustained activation. To test the
Inhibitors of Raf/MEK/ERK signaling (PD98059, U0126, and RAF kinase inhibitor 1) or JNK signaling (JNK inhibitor II) also did not decrease EGF activation of p38 (data not shown). In contrast, 1-h pretreatment with four different inhibitors of Src kinase, PP1 (1 μM), PP2 (1 μM), CGP77675 (2 μM), and SU6656 (500 nM), efficiently abrogated the ability of EGF to activate p38 in these cells (Fig. 8A). These compounds did not block tyrosine phosphorylation of the EGF receptor following addition of EGF. Additionally transient transfection of YAMC cells with a dominant-negative Src construct decreased EGF activation of p38 (Fig. 8B). Thus, p38 MAPK is activated in YAMC cells by EGF through a pathway dependent upon Src kinase or a Src family member. These data predict that a Src family member tyrosine kinase activity is also required for YAMC cell migration, as shown in Fig. 8C support this conclusion. Pretreatment with CGP77675 abolished EGF-stimulated cell migration and somewhat delayed basal migration. This finding is in agreement with our previous publication (52) in which the Src-directed tyrosine kinase inhibitor PD161430 inhibited intestinal epithelial cell migration stimulated by either tumor necrosis factor or EGF.

To investigate which Src family members might be responsible for EGF-induced p38 activation and stimulated migration, whole cell lysates from YAMC cells were subjected to Western blot analysis using antibodies specific for Src and Yes, and second messenger generation. The p38 activation pathway is independent of PI 3-kinase/Akt and phospholipase C-γ, that regulate EGF-stimulated p38 activation in intestinal epithelial cells.

**DISCUSSION**

This report shows that EGF stimulates intestinal epithelial cell migration in part by promoting sustained signaling through a pathway involving the EGF receptor, Src family kinases, and p38 MAPK. Enhanced motility requires the presence of ligand after stimulation of initial signal transduction pathways and second messenger generation. The p38 activation pathway is independent of PI 3-kinase/Akt and phospholipase C-γ/PKC signaling, which are also required for EGF-stimulated migration in these cells (31). Thus, EGF stimulates several parallel pathways leading to cell motility, all of which appear to be required for accelerated restitution in response to growth factor. Interestingly, however, p38 may also function in a unique “switching” role determining whether the output of EGF signaling in these cells is proliferation or migration.

EGF binding to the EGFR rapidly initiates a number of signal transduction pathways, including Ras/Raf/MEK/ERK, phospholipase C-γ/PKC, and PI 3-kinase/Akt, that regulate...
cellular function. Our previous studies have focused on these related second messenger molecules in the regulation of proliferation, migration, and apoptosis (31, 52, 56, 67, 68). A requirement for the continuous presence of EGFR ligand hours after the activation of these pathways in cell migration has not been reported. However, a similar requirement for EGFR-stimulated proliferation (16, 55) as well as the described role of constitutively active EGFR mutants in pathological conditions suggests that the molecular pathways activated by sustained EGFR ligation merit further investigation.

Studies from several other systems are in agreement with a role for p38 in epithelial cell migration. H-Ras-stimulated motility in breast epithelial cells is reportedly dependent upon p38-linked matrix metalloproteinase 2 secretion (69). p38 has been implicated in the in vivo corneal epithelial wound healing process (45), although there are conflicting reports in this regard (70). Furthermore p38 activation has been demonstrated in an intestinal epithelial wound healing model (47), although to our knowledge the current study is the first report showing a specific requirement for p38 in growth factor-stimulated intestinal epithelial wound healing. Also of particular interest is the comparison of our results with data from corneal epithelial cells in which p38 appears to be involved in basal wound healing (48). Intestinal epithelial cells, however, activate p38 after wounding (Fig. 4 and Ref. 47), although the effects on cell migration except in the presence of growth factors. This difference may result from a difference in available growth factors secreted by these two cell types. Two recent studies using corneal epithelial tissue wound models show that restitution in this system is largely dependent upon heparin-binding EGF-like growth factor release and phosphorylation of the EGF receptor (47); the extent to which basal restitution in a system relies on metalloproteinase-mediated EGF ligand release might correlate with dependence of basal migration on p38. In this regard, we have observed only a partial dependence on metalloproteinase activity for basal but not EGF-stimulated restitution in intestinal epithelial cells. Thus, the promigratory signaling cascade outlined in this report may be a general feature of epithelial migration. Alternatively it is possible that some of the differences between these results stem from incomplete inhibition of p38 and variation in the susceptibility of physiological ErbB ligand generation to p38 inhibition.

The dependence of EGF-stimulated p38 activation on Src family tyrosine kinase(s), but not PKC or PI 3-kinase, may reflect the activation by EGF of a general p38-stimulatory pathway used by several growth factors in different cell types. Other reports are in accord with this idea. One study of ATF2 regulation by growth factors in fibroblasts indicates that this transcription factor is stimulated by p38 in a pathway that is partially dependent on Src (71). Carbachol-induced blockade of chloride secretion in T84 colon cancer cells also utilizes a pathway involving both Src and p38 (41) as does acute neu differentiation factor-induced signaling in SKBR3 breast cancer cells (42). On the other hand, PKC-dependent p38 activation pathways have been reported in other systems, and this result was not observed in our studies. It is possible that p38 induction by mechanical stress (72), inflammation (73, 74), or some proapoptotic signals (43) use a different general set of pathways.

With regard to Src family kinase involvement in EGFR-mediated event.
activated p38 activation and intestinal epithelial cell migration, both Src and Fyn were phosphorylated on the homolog of human Src Tyr-416 following EGF treatment of YAMC cells (Fig. 8E), and the Src inhibitors used in this and other studies exhibit cross-inhibition of multiple Src family members (Yes, Fyn, Lck, and Lyn). The dominant-negative Src construct, which has dual mutations in the inactivation loop and the ATP-binding domain, may also cross-inhibit other family members given their structural similarities (75). Thus, while it is clear that Src family members are involved in EGF-stimulated p38 activity and cell migration in YAMC cells and Src appears to be the family member most robustly activated by EGF in this system (Fig. 8E), Fyn is also activated by EGF in intestinal epithelial cells (Fig. 8E) and has been implicated in cell migration in other systems such as squamous cell carcinoma invasion (65). Thus, the possibility that Fyn is also involved in this signaling pathway remains and is an active area of investigation.

The finding that the availability of p38 activity in intestinal epithelial cells can determine the outcome of EGFR signaling, resulting in migration if p38 is active and proliferation if it is not, appears to be novel. Signaling network modeling studies (76, 77) suggest that the various MAPK modules may consistently function in this sort of role in mammalian cells, providing state-dependent switches that integrate a broad array of upstream signals into discrete output events. Our study and one other in cornenal epithelial cells (78) suggest this is the case for p38 in determining migration versus proliferation in epithelial cells. Furthermore p38 has a well established permissive role in apoptosis and has for example recently been implicated in switching between proliferation and apoptosis in ErbB2/EGFR-overexpressing breast cancer cells (19). The question of potential cross-talk between p38 and other MAPK signaling modules at the signal integration level remains an interesting area for further investigation. In particular, potential cross-regulation with ERK1/ERK2-mediated pathways, which our results show to be involved in primarily proliferation rather than migration in intestinal epithelial cells, is an area of ongoing interest.

In summary, the data presented in this report establish a novel signaling pathway for EGF-stimulated intestinal epithelial cell migration involving Src- and/or Fyn-dependent activation of p38. This pathway is required for EGF-stimulated intestinal cell migration but not, to any substantial extent, basal wound restitution or HGF signaling despite the fact that p38 is activated by mechanical stress/wounding in this system. p38 activation by EGF requires the activity of a Src family member but not PI 3-kinase or PKC. Furthermore p38 activity in this system functions as a switch for EGF-stimulated signaling, determining its outcome as either migration (p38 active) or proliferation (p38 inactive). Understanding the molecular mechanisms of this switch may provide insight into the mechanism by which the cell selects among the diverse biological effects attributed to EGFR-initiated signals and integrates extracellular inputs to an appropriate cellular response.

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