Requirement of the epidermal growth factor receptor in renal epithelial cell proliferation and migration

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Zhuang, Shougang, Yujing Dang, and Rick G. Schnellmann. Requirement of the epidermal growth factor receptor (EGFR) in renal tubular cell proliferation and migration. Am J Physiol Renal Physiol 287:F365–F372, 2004. First published June 22, 2004; 10.1152/ajprenal.00035.2004.—We showed that renal proximal tubular cells (RPTC) can proliferate and migrate following plating and oxidant or mechanical injury in the absence of exogenous growth factors; however, the mechanisms of this response remain unclear. We examined whether epidermal growth factor signaling is activated following plating and mechanical injury and mediates RPTC proliferation and migration. EGFR, Akt, Erk1/2, and P38 were activated after plating and mechanical injury, and their phosphorylation was further enhanced by addition of exogenous EGF. Inactivation of the EGFR with the selective inhibitor AG-1478 completely blocked phosphorylation of EGFR, Akt, and Erk1/2 and blocked cell proliferation and migration following plating and injury. Inhibition of PI3K with LY-294002 blocked Akt phosphorylation and proliferation, whereas U-0126 blocked Erk1/2 phosphorylation but had no effect on proliferation. Furthermore, P38 was phosphorylated following mechanical injury and the p38 inhibitor SB-203580 blocked p38 phosphorylation and cell migration. In contrast, neither PI3K nor Erk1/2 inhibition blocked cell migration. These results show that EGFR activation is required for RPTC proliferation and migration and that proliferation is mediated by PI3K, whereas migration is mediated by p38.

renal proximal tubular cells; phosphoinositide-3-kinase; extracellular signaling-regulated kinase; p38

The kidney has the ability to completely recover from an ischemic or toxic insult. In patients and animals, renal proximal tubule cells (RPTC) that do not die or detach from the basement membrane are thought to contribute to the regeneration of the tubular epithelium and the restoration of overall renal function. RPTC migration, proliferation, and repair of physiological functions are three crucial processes needed for structural and functional regeneration of the nephron (26).

The molecular mechanisms that regulate renal tissue repair are not fully understood. Previous studies indicated that activation of growth factor receptors with exogenous growth factors promotes RPTC proliferation and migration (24). Other studies showed that the epidermal growth factor (EGF) receptor (EGFR) is upregulated following ischemia-reperfusion injury in rats, rabbits, and humans with acute renal failure (4, 18, 36, 43). In addition, RPTC EGFR phosphorylation status is increased in the early phase of renal injury (47). Heparin-binding EGF (HB-EGF), one EGFR ligand, has been identified in RPTC, and HB-EGF expression increased following renal injury (11). EGF is localized to the ascending limb of Henle and the distal convoluted tubule but not the proximal tubule in the healthy kidneys. However, in postischemic kidneys, EGF immunoreactivity was associated with proximal tubules, suggesting that EGF may migrate to the RPTC and contribute to repair following renal injury (43). Very recently, Wang et al. (45) examined the role of the EGFR in renal repair using mice with an EGFR point mutation that reduces receptor tyrosine kinase activity and found a profound decrease in the rate of both functional and structural recovery following acute renal failure induced by mercuric chloride. The above studies suggest that the EGFR is a critical regulator of the regeneration of RPTC.

The EGFR is a prototypical member of a larger superfamily of receptor tyrosine kinases that are cell-surface, membrane-spanning proteins with intrinsic tyrosine kinase activity (10). Ligand binding to the EGFR results in receptor dimerization, which leads to activation of the tyrosine kinase domains and autophosphorylation of the receptor. A number of autophosphorylation sites have been identified in the intracellular domain, which allow for specific binding of downstream signaling molecules. The five potential signaling molecules, phospholipase Cγ, phosphatidylinositol-3-kinase (PI3K), Ras-GTPase-activating proteins, tyrosine phosphatase, and Src kinase, have separate binding sites on the EGFR and can interact with the EGFR via their Src-homology-2 domains. These interactions activate different intracellular signal transduction pathways and, thereby, elicit various cellular responses including proliferation, survival, differentiation, and migration. Among the signaling enzymes mediating proliferation and migration are PI3K (42) and ERK1/2 (46), which lies in the Ras pathway (15). P38 is also activated after EGFR activation (14) and has been implicated in migration (40). Although these signaling pathways are involved in proliferation and migration of different cell types, EGFR activation and the signaling molecules linking EGFR activation to proliferation and migration in RPTC following renal injury have not been elucidated.

An in vitro model is needed that mimics the recent in vivo study in mice to study the activation and signaling of the EGFR that leads to RPTC proliferation and migration following renal injury. We observed previously that isolated renal proximal tubules proliferate and migrate in culture to form a confluent
monolayer in the absence of exogenous growth factors (28, 31). Furthermore, confluent RPTC subjected to mechanical and oxidant injury migrated, proliferated, and reached monolayer confluence in the absence of exogenous growth factors (7, 27). Mechanical injury is a commonly used model for the study of repair and regeneration in RPTC and many other cell types (1, 13, 41). The advantage of this model is the clear delineation of cell loss and the ability to examine cell migration and proliferation in defined areas. Consequently, we used the above models to determine the role of the EGFR and associated signaling pathways in RPTC migration and proliferation following plating and mechanical injury.

MATERIALS AND METHODS

Chemicals and reagents. Human-recombinant EGF and HB-EGF were obtained from R&D Systems (Minneapolis, MN). LY-294002 and U-0126 were purchased from Cell Signaling Technology (Beverly, MA). AG-1478 was obtained from Biomol (Plymouth Meeting, PA). All other chemicals were purchased from Sigma (St. Louis, MO). Antibodies to phospho-EGFR (2236), phospho-Akt (9271), Akt (2966), phospho-p38 (9211), p38 (9212), and phospho-ERK1/2 (9101) were obtained from Cell Signaling Technology. Antibodies to ERK1/2 (06-182) and EGFR (Sc-03-G) were purchased from BD Laboratories (San Diego, CA) and Santa Cruz Biotechnology (Santa Cruz, CA), respectively.

Isolation and culture of renal proximal tubules. Female New Zealand White rabbits were purchased from Myrtle’s Rabbitry (Thompson Station, TN). RPTC were isolated using the iron oxide perfusion method and grown in six-well tissue culture dishes under improved conditions as previously described (28, 29). The culture medium was a 1:1 mixture of DMEM/Ham’s F-12 (without glucose, phenol red, or sodium pyruvate) supplemented with 15 mM HEPES buffer, 2.5 mM l-glutamine, 1 mM pyridoxine HCl, 15 mM sodium bicarbonate, and 6 mM lactate. Hydrocortisone (50 nM), selenium (5 ng/ml), human transferrin (5 μg/ml), bovine insulin (10 nM), and l-ascorbic acid-2-phosphate (50 μM) were added daily to fresh culture medium.

Experimental protocols. To determine RPTC proliferation and migration following plating, isolated tubules were plated at 1 mg protein/well. On day 3, RPTC were incubated in the presence or absence of various pharmacological inhibitors for different time periods as indicated in the figure legends. To measure the rate of RPTC proliferation following mechanical injury, RPTC were grown to confluence (day 6) and monolayers were wounded by scraping cells off the plate with a hair comb to produce four 2-mm-wide swipes. Similar wounding was produced again perpendicular to the original swipe. Wound cultures were mock-wounded by moving the comb as described above through the medium, but without damaging the cell monolayer. After being washed once with PBS to remove cell debris, cells were incubated for 24 h in the presence or absence of various pharmacological inhibitors. To measure RPTC migration, RPTC were grown to confluence, and monolayers were wounded with a rubber policeman to produce a linear 4-mm swipe. After being washed once with PBS, cells were incubated for 24 h in medium in the presence of 0.5 μg/ml mitomycin C to block any proliferation. Cell migration was determined using a microscope and camera, and the wound area was calculated using National Institutes of Health Image software (1.6).

MTT assay. A 3-[4,5-di(methylthiazol-2-yl)-2,5-diphenyl-tetrazo- lium bromide (MTT) assay was used to assess cell proliferation (16, 20). After a 48-h exposure to various inhibitors or diluents, MTT was added (final concentration of 0.5 mg/ml) to individual cultures. RPTC were incubated for an additional 30 min, and tetrazolium was released by dimethyl sulfoxide. Optical density was determined with a spectrophotometer (570 nm). Data were normalized to solvent-treated cultures.

Cell cycle analysis. Cell cycle phase was determined using flow cytometry. Cells were harvested, stained with propidium iodide, and the number of cells in S-phase of the cell cycle was determined.

Preparation of cell lysates and immunoblot analysis. After different treatments, RPTC were washed twice with PBS without Ca²⁺ and Mg²⁺ and harvested in lysis buffer (0.25 M Tris-HCl pH 6.8, 4% SDS, 10% glycerol, 1 mg/ml bromophenol blue, and 0.5% 2-mercaptoethanol). Cells were disrupted by sonication for 15 s and lysates were stored at −20°C.

Equal amounts of cellular protein lysate were separated on 10% polyacrylamide gels and electrophoretically transferred to nitrocellulose membranes. After treatment with 5% skim milk at 4°C overnight, membranes were incubated with various antibodies for 1 h and then incubated with an appropriate horseradish peroxidase-conjugated secondary antibody (Amersham, Piscataway, NJ). Bound antibodies were visualized following chemiluminescence detection on autoradiographic film.

Statistical analysis. Data are presented as means ± SE and were subjected to one-way ANOVA. Multiple means were compared using Tukey’s test. P < 0.05 was considered a statistically significant difference between mean values. Renal proximal tubules isolated from an individual rabbit represent a single experiment (n = 1) consisting of data obtained from three wells.

RESULTS

EGFR mediates RPTC proliferation after plating, injury, and in response to EGF and HB-EGF. We previously made improvements in the culture of RPTC such that following plating, RPTC can migrate, proliferate, and reach confluence in the presence of exogenous growth factors (28, 29). Consequently, this RPTC model is ideal to examine the basis for the signaling of proliferation and migration in epithelial cells in the absence of exogenous growth factors. The first series of experiments examined whether the EGFR is required for cell proliferation following plating using the specific EGFR inhibitor AG-1478 (32). Three days after plating, increasing concentrations of AG-1478 were added and cell proliferation was determined 48 h later using the MTT assay. AG-1478 decreased RPTC proliferation in a concentration-dependent manner with maximal inhibition at 10 μM (Fig. 1A). With the use of the same experimental protocol, the addition of 10 ng/ml of exogenous EGF increased proliferation ~20% and AG-1478 blocked the exogenous EGF-mediated proliferation (Fig. 1B).

Similar experiments were conducted in which the number of cells in the S-phase of the cell cycle was used as a marker of proliferation. Approximately 22% of RPTC were in the S-phase 4 days following plating (Fig. 1C) and increased to ~29% following a 24-h exogenous EGF treatment. AG-1478 decreased the number of cells in the S-phase under basal conditions and following exogenous EGF exposure. Similar effects were observed using HB-EGF (Fig. 1D).

To determine whether the EGFR also mediates RPTC proliferation following injury, we examined the effect of AG-1478 on RPTC proliferation following mechanical injury. Confluent RPTC were subjected to mechanical injury and then incubated in the presence and absence of AG-1478 for 24 h. Confluent RPTC were growth arrested, with ~2% of the cells in S-phase (Fig. 1E). The number of RPTC in the S-phase increased to ~7% after mechanical injury and AG-1478 blocked the increase in the number of cells in the S-phase. Together, these data illustrate that the EGFR is critical in regulating RPTC proliferation following plating and mechanical injury in the absence.
of exogenous growth factors. In addition, exogenous EGF and HB-EGF are able to further stimulate the proliferation of RPTC.

**EGFR activation after RPTC plating, injury, and EGF exposure.** RPTC were cultured for 4 days and then incubated in the absence and presence of 10 ng/ml EGF for 0–60 min. EGFR phosphorylation was measured using immunoblot analysis with an antibody that recognizes the phosphorylation of the EGFR at tyrosine 1068 (22). Total EGFR content was measured using immunoblot analysis and an antibody that recognizes the EGFR independent of its phosphorylation state. EGFR phosphorylation (p-EGFR) was observed in RPTC undergoing proliferation, was increased in the presence of exogenous EGF within 5 min, and was maintained at the elevated level for 30 min (Fig. 2A). Treatment with AG-1478 completely blocked EGFR phosphorylation (Fig. 2B). Total EGFR levels did not change under these experimental conditions.

Low levels of EGFR phosphorylation were observed in nonwounded confluent monolayers (Fig. 2C). EGFR phosphorylation increased within 10 min of mechanical injury, remained elevated through 60 min, and was blocked by AG-1478. Total EGFR levels did not change under these experimental conditions. These data illustrate that the EGFR is activated (i.e., phosphorylated) during proliferation following plating in the absence of exogenous growth factors, in confluent RPTC, and following mechanical injury. Furthermore, exogenous EGF further activated the EGFR and AG-1478 completely blocked EGFR phosphorylation.

**EGFR mediates activation of Akt and ERK1/2 following RPTC plating and mechanical injury.** The PI3K/Akt and ERK pathways mediate cell proliferation in different cell types, and these pathways can be activated in response to EGFR phosphorylation (34). We evaluated whether the PI3K/Akt and/or ERK1/2 pathways are activated following RPTC plating in the absence and presence of exogenous EGF, in confluent RPTC, and following mechanical injury. Furthermore, we determined whether the EGFR was responsible for the activation of PI3K/Akt and/or ERK1/2. The activation of the PI3K and ERK1/2 pathways was measured using immunoblot analysis and antibodies that recognize phosphorylated Akt (p-Akt; a target of PI3K) and ERK1/2 (p-ERK1/2), respectively. Total Akt and ERK1/2 content was measured using immunoblot analysis and antibodies that recognize the Akt and ERK1/2 independent of their phosphorylation state. Akt and ERK1/2 phosphorylation was observed in RPTC undergoing proliferation, was enhanced in the presence of EGF within 5 min, and was blocked by the EGFR inhibitor AG-1478 at 10 min and 24 h (Fig. 3A, B, E). Low levels of Akt and ERK1/2 phosphorylation were observed in confluent RPTC (Fig. 3C). After mechanical injury, Akt and ERK1/2 phosphorylation increased within 5 min, was maintained through 60 min, and was blocked by AG-1478 (Fig. 3, C and D). These data illustrate that the PI3K and ERK1/2 pathways are activated during RPTC proliferation after plating and following mechanical injury. Furthermore, the EGFR is responsible for the activation of PI3K and ERK1/2 under these conditions. Exogenous EGF further activated PI3K and ERK1/2.
Activation of PI3K/Akt pathway, but not ERK1/2, is required for RPTC proliferation following plating and mechanical injury. To determine whether PI3K/Akt and/or ERK1/2 activation mediates RPTC proliferation following plating and mechanical injury, RPTCs were assayed for proliferation in the presence and absence of the PI3K inhibitor LY-294002 (44) and the MEK inhibitor U-0126 (8). Inhibition of PI3K decreased the number of RPTC in the S-phase following plating and mechanical injury (Fig. 4A). Inhibition of PI3K decreased the number of RPTC in the S-phase to control levels following plating in the presence of exogenous EGF. In contrast, inhibition of MEK did not decrease the number of RPTC in the S-phase following plating in the presence and absence of exogenous EGF or following injury.

To confirm that LY-294002 and U-0126 selectively inhibited their respective kinases, activation of Akt and ERK1/2 was monitored using immunoblot analysis and antibodies that recognize phosphorylated and total Akt and ERK1/2 as described above. LY-294002 blocked Akt phosphorylation and U-0126 blocked ERK1/2 phosphorylation of RPTC following plating in the presence and absence of exogenous EGF at 10 min and 24 h (Fig. 4C, E, and F). Similarly, these two inhibitors also abolished phosphorylation of Akt and ERK1/2 at 5 min following mechanical injury (Fig. 4D). LY-294002 had no effect on ERK1/2 phosphorylation and U-0126 had no effect on Akt phosphorylation under the same conditions. These data strongly suggest that although both PI3/Akt and ERK pathways are activated under these experimental conditions, PI3K is responsible for RPTC proliferation.

EGFR mediates RPTC migration following plating, injury, and in response to EGF. To elucidate the role of the EGFR in RPTC migration following injury, confluent RPTC were mechanically injured and then incubated for 24 h in the absence or presence of AG-1478 and/or exogenous EGF. RPTC migrated into the denuded area and partially filled the wound area 24 h following mechanical injury (Fig. 5). Exogenous EGF enhanced wound closure while RPTC migration was blocked by AG-1478 in the presence or absence of exogenous EGF. These observations suggest that the EGFR mediates RPTC migration following injury and in response to EGF.

p38, but not PI3K and ERK, pathways mediate RPTC migration. It has been reported that ERK, PI3K, and p38 mediate cell migration in different cell types (35). To determine whether RPTC migration following mechanical injury

![Fig. 2. AG-1478 inhibits EGFR phosphorylation following plating, EGF exposure, and mechanical injury.](image)

![Fig. 3. Effect of AG-1478 on Akt and ERK1/2 phosphorylation following plating, EGF exposure, and mechanical injury.](image)
was mediated by ERK1/2, p38, and/or PI3K pathways, inhibitors of ERK1/2 (U-0126), Akt (LY-294002), or p38 (SB-203580) were added immediately before mechanical injury, and cell migration was determined 24 h later. RPTC migration was inhibited by SB-203580, but not U-0126 or LY-294002, suggesting that p38, but neither ERK1/2 nor PI3K, mediates RPTC migration (Fig. 6A). The effectiveness of LY-294002 and U-0126 in inhibiting Akt and ERK1/2 was shown in Fig. 4D. To determine whether p38 activation was induced following injury, RPTC were subjected to mechanical injury and then immunoblot analysis using an antibody that specifically recognizes phosphorylated p38. p38 is constitutively phosphorylated in confluent RPTC, and its phosphorylation increased within 5 min following mechanical injury (Fig. 6B). Total p38 protein levels did not change over the experimental period. Treatment of cells with SB-203580 blocked p38 phosphorylation under basal and following injury (Fig. 6D). In the presence of AG-1478, mechanical injury-induced p38 phosphorylation was also inhibited (Fig. 6D). These data suggest that the EGFR mediates p38 activation following injury, but the constitutive activation of this kinase is independent of EGFR.

DISCUSSION

Previous studies showed that RPTC can migrate, proliferate, and repair physiological functions following oxidant, toxicant, and mechanical injury in the absence of exogenous growth factors (7, 27, 29). Furthermore, exogenous EGF stimulated migration and proliferation following oxidant and mechanical injury (2, 30, 41). However, the physiological role of EGFR in these RPTC-regenerative processes is still not clear. In this study, we demonstrated that the EGFR is constitutively activated following plating in the absence of exogenous growth factors and is further stimulated by mechanical injury. Furthermore, the specific EGFR inhibitor AG-1478 blocked EGFR activation and blocked RPTC proliferation and migration following mechanical injury. AG-1478 decreased but did not completely block RPTC proliferation following plating. These results show that EGFR is indispensable for RPTC proliferation and migration and clearly assign the EGFR to the regulation of RPTC-regenerative responses. Despite the importance of EGFR in mediating RPTC proliferation and migration, it is possible that other growth factor receptors may also play a role because AG-1478 did not fully inhibit RPTC proliferation following plating.

These results are in agreement with the recent findings of Wang et al. (45), who observed that mice containing a mutation in the EGFR that results in reduced receptor kinase activity exhibited less renal DNA synthesis than wild-type mice treated with mercuric chloride (6). Furthermore, although the acute renal failure resolved over 6 days in wild-type mice, the acute renal failure in the mice containing the mutation in the EGFR did not resolve.

The mechanism by which EGFR is activated following plating and mechanical injury remains to be studied. In the absence of an influx of an EGFR ligand from a distal site in vivo, EGFR activation has been thought to be associated with autocrine/paracrine secretion of soluble ligands (33). Because our culture system does not contain serum or EGF ligands, it is possible that EGFR activation following plating and injury
may be induced by an autocrine mechanism. In this regard, multiple growth factors including EGF, transforming growth factor-β, HB-EGF, amphiregulin, and batacellulin serve as EGFR ligands (9). Among them, HB-EGF is expressed in RPTC and exogenous HB-EGF is able to stimulate RPTC proliferation (11) (Fig. 1). Furthermore, it has been reported that HB-EGF expression is increased following renal ischemia-reperfusion injury (11). A previous study confirmed the existence of an autocrine EGFR loop with HB-EGF as one of its ligands in the wounding healing of keratinocytes (21). Thus it will be interesting to examine whether HB-EGF plays an autocrine role in RPTC proliferation and migration.

Alternatively, EGFR may also be activated via a ligand-independent mechanism, involving cell adhesion to the extracellular matrix. Moro et al. (23) showed that EGFR can be activated following cell plating on various extracellular substrates that bind integrins. Furthermore, adhesion to the matrix induces the formation of a macromolecular complex containing β3-integrin, EGFR, Src, and p130 cas (22). Matrix adhesion and Src activity are required for the assembly of this complex that leads to EGFR phosphorylation (22). Other studies by Arias-Salgado et al. (3) demonstrated that Src can directly interact with β3-integrin, and clustering of β3-integrin induces Src phosphorylation. These studies support the possibility that integrin-mediated cell adhesion induces cell proliferation and migration via EGFR. In this context, we showed previously that addition of the integrin substrate collagen IV to culture medium promotes functional recovery of RPTC, whereas interruption of collagen IV deposition decreases this response (25). Although it remains unclear whether collagen IV-triggered activation of integrin is involved in EGFR activation and

### Figure 5: Effect of AG-1478 (AG) on RPTC migration following mechanical injury and EGF exposure

Confluent monolayers of RPTC were scraped to create mechanical injury and incubated for 24 h in the presence and absence of 10 ng/ml EGF and/or 10 μM AG. A: photographs at ×40 magnification. B: migration into the wounded area was quantified by measuring the migration of cells from the wound edge. Each bar represents means ± SE, n > 8. Bars with different superscripts are significantly different from one another (P < 0.05).

### Figure 6: Role of P38, ERK1/2, and p38 in RPTC migration following mechanical injury

Confluent monolayers of RPTC were either mock-scraped or scraped to create mechanical injury and then incubated for 24 h in the presence or absence of 20 μM AG-1478 (AG), 20 μM U-0126, or 20 μM LY. A: migration into the wounded area was quantified by measuring the migration of cells from the wound edge. Each bar represents means ± SE, n > 8. Bars with different superscripts are significantly different from one another (P < 0.05). Confluent monolayers of RPTC were scraped to produce mechanical injury (injury) and then incubated for 0–60 min (B) or pretreated with 20 μM AG-1478 (D) for 1 h and then incubated for 5 min following mechanical injury. RPTCs were subjected to immunoblot analysis using antibodies to phosphorylated p38, and protein loading was monitored using an antibody to total p38 protein.
proliferation of renal epithelial cells, Sanders and Basson (38) reported that the migration of intestinal epithelial cells on collagen IV needs integrin-mediated Src activation. Taken together, EGFR activation may be induced by the ligand-dependent and/or -independent mechanisms in renal regeneration.

Although RPTC EGFR activation occurs in the absence of exogenous growth factors, addition of exogenous EGF further enhanced EGFR phosphorylation as well as RPTC proliferation and migration, suggesting that the EGFR has a potential to respond to additional stimulation. Consistent with our observation, Moro et al. (23) observed that exogenous EGF further stimulated EGFR phosphorylation in cells seeded on fibronectin. These findings support the hypothesis that cell adhesion is probably a priming step for EGFR activation, but full activation of EGFR needs ligand stimulation (23). Although the mechanisms underlying cell-extracellular matrix interactions that facilitate a response to growth factors are still not clear, this phenomenon may have important relevance to renal regeneration following injury. Assuming that paracrine and endocrine production and transport of EGF ligands occur following in vivo renal injury, further EGFR stimulation by these EGF ligands would strengthen the regenerative responses. In addition, this may also account for the effectiveness of exogenous EGF in accelerating renal regeneration as demonstrated in in vivo and in vitro experiments (6, 12, 30, 41).

The biological functions elicited by EGFR activation are mediated by different signaling pathways. In this study, we show that the PI3K/Akt pathway is downstream of EGFR and is required for RPTC proliferation following plating and mechanical injury and in response to exogenous EGF. Although ERK1/2 was also phosphorylated following RPTC plating and mechanical injury, inhibition of this pathway by U-0126 did not result in a significant effect on RPTC proliferation, suggesting that the ERK1/2 pathway is not involved in EGFR-mediated proliferation. The functional significance of ERK activation in RPTC remains unclear and needs further investigation.

Although EGFR activation also mediates RPTC migration, the PI3K and ERK1/2 pathways are not involved in this response. In contrast, we found that p38 is activated following mechanical injury, is downstream of EGFR, and blockade of p38 with SB-203580 inhibited migration, strongly suggesting that p38 is an important signaling molecule of RPTC migration. Furthermore, the p38 inhibitor also blocked HSP-27 phosphorylation (data not shown), a protein that is associated with the formation of stress fibers and focal adhesions in cell migration (39). How EGFR activation is coupled to a signaling pathway leading to p38 following mechanical injury is not clear. No direct relationship between EGFR and p38 has been reported. A likely mechanism is that EGFR regulates an upstream activator in the p38 cascade. For example, it has been reported that the EGFR mediates Cdc42 (5) and Rac (17) activation, both of which are upstream activators of p38 (19) with links to cell migration (37).

In summary, this study is the first to link EGFR with two tissue-repair responses in renal epithelial cells and to identify EGFR activation as a potential mechanism to control RPTC proliferation and migration in the absence of exogenous growth factors. Proliferation through EGFR was associated with PI3K/Akt signaling, whereas migration induced by EGFR activation was associated with p38 signaling.

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