p38 Kinase-mediated Transactivation of the Epidermal Growth Factor Receptor Is Required for Dedifferentiation of Renal Epithelial Cells after Oxidant Injury*

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Renal proximal tubular cell (RPTC) dedifferentiation is thought to be a prerequisite for regenerative proliferation and migration after renal injury. However, the specific mediators and the mechanisms that regulate RPTC dedifferentiation have not been elucidated. Because epidermal growth factor (EGF) receptor activity is required for recovery from acute renal failure, we examined the role of the EGF receptor in dedifferentiation and the mechanisms of EGF receptor transactivation in primary cultures of RPTCs after oxidant injury. Exposure of confluent RPTCs to H2O2 resulted in 40% cell death, and surviving RPTCs acquired a dedifferentiated phenotype (e.g. elongated morphology and vimentin expression). The EGF receptor, p38, Src, and MMK3 were activated after oxidant injury and inhibition of the EGF receptor or p38 with specific inhibitors (AG1478 and SB203580, respectively) blocked RPTC dedifferentiation. Treatment with SB203580 or adenoviral overexpression of dominant negative p38α or its upstream activator, MMK3, inhibited EGF receptor phosphorylation induced by oxidant injury, whereas AG1478 had no effect on p38 phosphorylation. Inhibition of Src with 4-amino-5-(4-methylphenyl)-7-(t-butyl)pyrazolo[3,4-d]-pyrimidine (PP1) blocked MMK3 and p38 activation, and inhibition of MMK3 blocked p38 activation. In addition, inactivation of Src, MMK3, p38, or the EGF receptor blocked tyrosine phosphorylation of β-catenin, a key signaling intermediate that is involved in the epithelial-mesenchymal transition and vimentin expression. These results reveal that p38 mediates EGF receptor activation after oxidant injury; that Src activates MMK3, which, in turn, activates p38; and that the EGF receptor signaling pathway plays a critical role in RPTC dedifferentiation.

In many cases, kidneys subjected to an ischemic or toxic insult can completely recover. For example, surviving RPTCs

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‡ The abbreviations used are: RPTC, renal proximal tubular cell; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; JNK, c-Jun NH2-terminal kinase; MMK, mitogen-activated protein kinase kinase kinase; PP1, 4-amino-5-(4-methylphenyl)-7-(t-butyl)pyrazolo[3,4-d]-pyrimidine; MOI, multiplicity of infection; ERK, extracellular signal-regulated kinase; X-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; pfu, plaque-forming unit(s); p-, phospho.

are thought to be crucial for the restoration of renal function after acute renal failure. During the recovery process, surviving RPTCs convert to a dedifferentiated phenotype and then proliferate and migrate to replace lost cells (1, 2). Finally, regenerating RPTCs differentiate and resume normal structure and functions. Because dedifferentiation of RPTCs is a prerequisite for migration and proliferation, it is important to understand the molecular mechanisms of RPTC dedifferentiation. In turn, this information may contribute to the development of novel treatments that stimulate RPTC regeneration and decrease recovery time of acute renal failure.

Dedifferentiation is a process by which differentiated cells reverse the normal developmental processes and become mesenchymal-like proliferating cells. Epithelial cells in the adult kidney are usually cuboidal in shape with a high degree of apical-basal polarity. In contrast, mesenchymal cells are elongated or stellate in shape and are characterized by expression of vimentin intermediate filaments (3, 4). In vivo studies demonstrated that in response to ischemia/reoxygenation injury, RPTCs express vimentin, which was detected within 1 day and absent by 16 days after ischemia/reperfusion injury (5). Concomitant with vimentin expression, proliferating cell nuclear antigen (a marker of proliferating cells) is positive in these cells (5). Thus, a dedifferentiation response occurs in surviving RPTCs after injury that is followed by proliferation and return of normal RPTC morphology and function.

Because the regeneration process recapitulates the developmental paradigm in numerous ways, the mediators mediating both processes may be similar. For example, Devarajan et al. (6) provided evidence that some genes that are known to play a key role during early renal morphogenesis also are induced within 3 h of renal ischemia/reperfusion injury. p38 kinase is an important signaling molecule in kidney development. Its expression is predominantly distributed in mesenchymal cells and ureteric bud epithelia of the fetal kidney (7, 8), and pharmacological inhibition of p38 in embryonic explants inhibits kidney growth (7). In addition, an increase in p38 activity was associated with a switch to a mesenchymal-like cell phenotype in transforming growth factor β-stimulated renal epithelial cells (9). p38 kinases belong to the mitogen-activated protein kinase superfamily, which consists of ERK1/2, JNK, and p38 (10). Currently, four different p38 isoforms (p38α, p38β, p38γ, and p38δ) have been identified, and p38α and p38β are inhibited specifically by the pyridinyl imidazole SB203580 (11). MMK3 and MMK6 are upstream activators of p38 kinases (12).
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opment and tubulogenesis (13–15). The addition of tyrosine kinase inhibitors and EGF receptor-blocking antibodies to metanephric cultures blocks differentiation of the ureteric bud (15). Furthermore, homozygous mice engineered with a targeted disruption of the EGF receptor exhibit abnormalities in the differentiation of structures derived from the ureteric bud (16).

There is in vivo and in vitro evidence that the EGF receptor is an important regulator of RPTC regeneration after injury. It is up-regulated in rats and rabbits after ischemia/reperfusion injury and in humans with acute renal failure (17–19). Activation of the EGF receptor with exogenous EGF promotes RPTC proliferation and migration (20, 21), and inhibition of the EGF receptor blocks RPTC proliferation and migration (22). Wang and co-workers (23) recently found a profound decrease in the rate of functional and structural recovery of the tubular epithelium after acute renal failure induced by mercuric chloride in mice with an EGF receptor point mutation that reduces the receptor tyrosine kinase activity.

The EGF receptor can be activated by different stimuli through diverse mechanisms. Ligands binding to the EGF receptor result in receptor dimerization, activation of tyrosine kinase domains, and autophosphorylation of the receptor (24). In addition, nonspecific stimuli such as osmotic stress, ultraviolet light, oxidative stress, and hypoxia/reoxygenation injury and G-protein-coupled receptor stimulation also trigger EGF receptor autophosphorylation (24–28). The activation of a cellular membrane receptor by a stimulus other than its ligand is termed receptor transactivation. Activation of the EGF receptor leads to phosphorylation of several tyrosine sites in the intracellular domain, which allows for specific binding of downstream signaling molecules and results in various cellular responses.

As a member of cell to cell drop adherence junctions and the ability to link E-cadherin to the cytoskeleton, β-catenin plays an essential role in intercellular adhesion (9). β-Catenin has been identified as a signaling target of the EGF receptor (29), and its activation is associated with epithelial-mesenchymal transition (30). Tyrosine phosphorylation of β-catenin leads to dissociation of the E-cadherin-β-catenin complex, nuclear translocation of β-catenin, and concomitant induction of TCF target genes (31). β-Catenin can drive the gene expression necessary for induction of the epithelial-mesenchymal transition such as Slug (32, 33) and the dedifferentiation marker, vimentin (34). Exposure of epithelial cells to oxidants also leads to phosphorylation of β-catenin and disruption of epithelial cell-cell contacts and translocation of β-catenin into nucleus (35, 36). In this study, we investigated the mechanisms by which the EGF receptor is activated after injury and its role in RPTC dedifferentiation.

MATERIALS AND METHODS

Reagents—Human recombinant EGF was purchased from R&D Systems (Minneapolis, MN). LY294002 and AG1478 were obtained from Cell Signaling Technology (Beverly, MA) and Biomol (Plymouth Meeting, PA), respectively. All other chemicals were purchased from Sigma. Antibodies to phospho-EGF receptor (Tyrr1068), phospho-Akt, Akt, phospho-Src (Tyrr416), Src, phospho-p38, p38, and phospho-ERK1/2 were obtained from Cell Signaling Technology. Antibodies to ERK1/2 and EGF receptor were purchased from BD Laboratories (San Diego, CA) and Santa Cruz Biotechnology (Santa Cruz, CA), respectively. All antibodies were used at 1:1000 for immunoblotting.

Isolation and Culture of Renal Proximal Tubules—Female New Zealand White rabbits (2 kg) were purchased from Myrtle’s Rabbitry (Thompson Station, TN). RPTCs were isolated using the iron oxide perfusion method and grown in 6-well or 35-mm tissue culture dishes under improved conditions as previously described (37). The culture medium was a 1:1 mixture of Dulbecco’s modified Eagle’s medium/Ham’s F-12 (without glucose, phenol red, or sodium pyruvate) supplemented with 15 mM HEPES buffer, 2.5 mM l-glutamine, 1 mM pyruvate, 15 mM sodium bicarbonate, and 6 mM lactate. Hydrocortisone (50 nM), selenium (5 ng/ml), human transferrin (5 μg/ml), bovine insulin (10 μU), and L-ascorbic acid-2-phosphate (50 μM) were added daily to fresh culture medium.

Confluent RPTCs were used for all experiments. RPTC monolayers were pretreated with various inhibitors for 1 h and exposed to 1 mM H₂O₂ for different periods of time as indicated in the figure legends. For some experiments, RPTCs were treated with H₂O₂ for 5 h, washed twice with culture medium, and then incubated for an additional 24 h in the presence or absence of various inhibitors. All experiments were repeated at least three times, and representative results are illustrated in the figures.

Replication-deficient Adenovirus Infection—Recombinant adenovirus expressing kinase inactive form of human p38 (Ad-p38δdn) and wild type (Ad-p38α) were propagated in HEK293 cells using standard methods. Transient transfections of HEK293 cells were performed using Lipofectamine 2000 (Invitrogen). Viral titers were determined by plaque formation assay using HEK293 cells and expressed as plaque-forming units (pfu). RPTCs were infected with each virus at a multiplicity of infection (MOI) of 100 pfu for 2 h at 37 °C in a humidified, 5% CO₂ incubator. Afterward, the cultures were placed in normal culture media for an additional 48 h and then exposed to oxidant injury for the time periods described in the figure legends. At an MOI of 100 pfu, 100% of the cells showed expression of the viral gene insert as indicated by X-gal assay. A viral assay of RPTCs infected with recombinant adenovirus expressing lacZ (data not shown).

Preparation of Cell Lysates and Immunoblot Analysis—After treatment, RPTCs were washed twice with phosphate-buffered saline 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. Equal amounts of cellular protein lysates were separated by SDS-PAGE, 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 Biosciences) for 1 h. Bound antibodies were visualized after chemiluminescence detection on autoradiographic film.

Results

p38-mediated EGF Receptor Activation Is Required for RPTC Dedifferentiation—Dedifferentiation of epithelial cells is characterized by the conversion of epithelial cells to a fibrolast-like phenotype, the epithelial-mesenchyme transition (4). To understand the mechanism of RPTC dedifferentiation, we determined whether the EGF receptor is required for formation of this phenotype after oxidant injury. Confluent RPTCs were exposed to H₂O₂ for 5 h and then incubated for 24 h in the absence or presence of AG1478, a specific inhibitor of the EGF receptor (38). After oxidant injury, ~40% of the RPTCs died as indicated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT) (data not shown). The remaining cells became elongated or stellate in shape, a feature of dedifferentiation (Fig. 1B). In the presence of AG1478, RPTCs retained the epithelial cell morphology (Fig. 1C).

Previous in vivo and in vitro studies showed that vimentin, a filament protein that is expressed in mesenchymal cells but not in mature nephron epithelium, is detectable in RPTCs after ischemia/reperfusion (5) and after toxicant injury in RPTCs (39). Therefore, we examined vimentin expression in RPTCs after oxidant injury in the presence or absence of AG1478. Whereas vimentin was not detected in confluent RPTCs by
Inhibition of the p38 Pathway, but Not the ERK1/2 Pathway, Blocks RPTC Dedifferentiation—Activation of p38 and ERK1/2 signaling pathways is implicated in the epithelial-mesenchymal transition in renal cells (40, 41). We determined whether the p38 and ERK1/2 pathways mediate RPTC dedifferentiation. RPTCs were exposed to 1 mM H2O2 for 5 h and then incubated in fresh culture medium for 24 h in the presence or absence of 10 μM AG1478, 20 μM SB203580, and 10 μM U0126. After fixation, the cells were stained with anti-vimentin antibody. Representative micrographs are shown. A, control confluent RPTCs; B, H2O2; C, H2O2 + AG1478; D, H2O2 + SB203580; E, H2O2 + U0126.

H2O2-Induced Phosphorylation of the EGF Receptor and p38—Our previous studies showed that H2O2 induces EGF receptor activation, which is required for phosphorylation of ERK1/2, but not Akt in RPTCs (43). We examined p38 activation after H2O2 exposure and the relationship between p38 and EGF receptor activation. RPTCs were exposed to H2O2 in the presence or absence of AG1478, and the phosphorylation of each protein was determined using immunoblot analysis and antibodies that recognize the phosphorylated EGF receptor and p38. After H2O2 exposure, EGF receptor phosphorylation increased at 10 min, reached maximum levels at 30 min, and was persistent for at least 60 min (Fig. 2A). H2O2-induced p38 increased and was maximal in 5–10 min and returned to the basal level at 60 min. Although JNK kinases are also stress-responsive proteins, they were not activated in response to oxidant injury as assessed by immunoblot analysis and an anti-phosphorylated JNK antibody (data not shown). AG1478 completely blocked H2O2-induced EGF receptor phosphorylation but did not affect p38 phosphorylation (Fig. 2B). We suggest that p38 is activated after oxidant injury and that the EGF receptor is not responsible for p38 activation under this condition.

p38 Activation Is Required for EGF Receptor Phosphorylation after H2O2 Exposure—To examine whether p38 is required for EGF receptor activation in response to oxidant injury, RPTCs were pretreated with the p38 inhibitor SB203580 and then exposed to H2O2. SB203580 blocked H2O2-induced EGF receptor and p38 phosphorylation (Fig. 3A). In contrast, inhibition of the phosphoinositide 3-kinase/Akt or ERK1/2 pathway did not affect H2O2-induced phosphorylation of the EGF receptor (Fig. 3, B and C). We suggest that p38 is responsible for EGF receptor activation after oxidant injury.

p38 Is Required for EGF Receptor Phosphorylation Induced by Osmotic Stress and UV Light, but Not EGF—p38 is activated by a number of other stimuli, including UV light and hyperosmotic shock (12). These stresses also have been shown to stimulate activation of the EGF receptor (26, 44). To examine whether p38 is responsible for EGF receptor activation by these stimuli, RPTCs were pretreated with SB203580 and then exposed to 1 mM NaCl and 500 J/m2 UV light. RPTCs treated with EGF were used as control. Inhibition of p38 blocked phosphorylation of the EGF receptor induced by NaCl or UV light (Fig. 4, A and B). In contrast, SB203580 had no effect on EGF receptor phosphorylation in EGF-treated RPTCs (Fig. 4C). These data are consistent with the above observations that p38 is specifically involved in EGF receptor activation induced by stress.

Overexpression of p38adn Inhibits EGF Receptor Phosphorylation after Oxidant Injury—To verify the role of p38 in...
H₂O₂-dependent EGF receptor activation, RPTCs were transfected with an adenovirus vector encoding a kinase-deficient human p38\(^{\text{H9251}}\) mutant (Ad-p38\(^{\text{H9251}}\)dn) (10). Adenovirus encoding LacZ was used as control. Ad-p38\(^{\text{H9251}}\)dn transfection decreased H₂O₂-induced EGF receptor phosphorylation to 38% of RPTCs transfected with the same amount of adenovirus encoding LacZ (Fig. 5, A and B). Inhibition of p38 activation by the p38\(^{\text{H9251}}\) mutant was confirmed using immunoblot analysis and an antibody against phospho-p38 (Fig. 5A). X-gal staining showed that 100% of RPTCs were infected by adenovirus using this protocol (data not shown).

Inhibition of MKK3 Blocks p38 and EGF Receptor Phosphorylation Induced by Oxidant Injury—MKK3 functions as an upstream activator of p38. Therefore, we examined the effect of MKK3 inhibition on EGF receptor and p38 activation induced by H₂O₂. H₂O₂-induced EGF receptor phosphorylation of MKK3, similar to the time course of p38 activation (Figs. 2 and 6A). Transfection of RPTCs with an adenovirus vector encoding a kinase-deficient human MKK3 mutant (Ad-MKK3dn) resulted in complete inhibition of p38 and EGF receptor phosphorylation in H₂O₂-treated cells (Fig. 6B). Transfection of RPTCs with an adenovirus encoding LacZ did not have any effect on oxidant-induced p38 and EGF receptor phosphorylation (Fig. 6B). These data support a role for MKK3 in p38 activation in response to oxidant injury.

p38 and MKK3 Act Downstream of Src in Mediating EGF Receptor Phosphorylation—Our previous studies indicated that Src mediates EGF receptor phosphorylation in response to H₂O₂ stimulation (43). To examine the relationship among p38, MKK3, and Src in EGF receptor phosphorylation after oxidant exposure, RPTCs were treated with diluent, the p38 inhibitor SB203580, or the Src inhibitor PP1 for 1 h and then exposed to H₂O₂. Cell lysates were analyzed by immunoblotting with antibodies to p-EGFR, EGFR, p-p38, p38, p-Akt, Akt, p-ERK1/2, and ERK1/2. Representative blots are shown.

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### FIG. 3
The p38 pathway, but not the ERK1/2 or phosphoinositide 3-kinase/Akt pathway, mediates oxidant-induced EGF receptor phosphorylation. Confluent RPTCs were pretreated with 20 μM SB203580 (SB) (A), 20 μM LY294002 (LY) (B), or 10 μM U0126 (C) for 1 h and then exposed to 1 mM H₂O₂ for 10 min. Cell lysates were analyzed by immunoblotting with antibodies to p-EGFR, EGFR, p-p38, p38, p-Akt, Akt, p-ERK1/2, and ERK1/2. Representative blots are shown.

### FIG. 4
p38 is required for osmotic stress- and UVC-induced (but not EGF-induced) EGF receptor activation. Confluent RPTCs were pretreated with 20 μM SB203580 (SB) for 1 h and then exposed to 1 mM NaCl (A), 500 J/m² UVC (B), or 10 ng/ml EGF (C) for 10 min. Immunoblot analysis was performed using antibodies to p-EGFR, EGFR, p-p38, and p38. Representative blots are shown.

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ing is responsible for the activation of β-catenin after oxidant injury.

p38, MMK3, and Src Mediate β-Catenin Activation after H₂O₂ Exposure—Because the above studies suggested that Src-mediated activation of p38 is coupled to EGFR phosphorylation after oxidant injury, we further examined whether p38, MMK3, and Src mediate tyrosine phosphorylation of β-catenin. As shown in Fig. 9A, pretreatment of cells with SB203580 blocked β-catenin tyrosine phosphorylation. Furthermore, transfection of RPTCs with an adenovirus vector encoding a kinase-deficient human p38 or MMK3 mutant inhibited β-catenin phosphorylation in H₂O₂-treated cells (Fig. 9, B and C). Transfection of RPTCs with an adenovirus encoding LacZ did not have any effect on oxidant-induced β-catenin phosphorylation. In addition, inhibition of Src by PP1 blocked β-catenin phosphorylation in response to oxidant injury. These data are consistent with the role of the EGF receptor in mediating β-catenin tyrosine phosphorylation and demonstrate that Src, MMK3, and p38 are in the same pathway and are required for this response after oxidant injury.

DISCUSSION

Our studies have shown that the EGF receptor mediates RPTC proliferation and migration after mechanical injury, plating of renal proximal tubules (22), and H₂O₂-induced EGFR receptor activation (43). We suggested that the EGF receptor is critical for the regenerative response in renal epithelial cells and can be activated in response to oxidant injury. Because conversion of epithelia to the dedifferentiated phenotype is an early step in renal generation after injury, we examined the
p38-mediated EGF receptor activation is specific to stress stimuli because activation of the EGF receptor with osmotic stress or UVC also required p38 activity, whereas EGF-induced EGF receptor phosphorylation did not. Whereas JNK is activated in response to oxidant stress in some systems, it is not activated by H$_2$O$_2$ in RPTCs as determined by immunoblot analysis and specific anti-phosphorylated antibodies (data not shown), suggesting that it may not be involved in the regulation of EGF receptor activation in this cell type. In addition, phosphoinositide 3-kinase/Akt and ERK pathways do not mediate EGF receptor phosphorylation, although these two pathways are activated in response to oxidant injury (43).

Our data also revealed that multiple isoforms of p38 may be involved in the transactivation of the EGF receptor after oxidant injury. p38$\alpha$ and p38$\beta$ are ubiquitously expressed in tissues and are reported to be specifically inhibited by SB203580 (47). However, treatment with this inhibitor did not abolish EGF receptor phosphorylation after oxidant injury. In contrast, overexpression of dominant negative mutants of MKK3 resulted in complete inhibition of this event. Given that MKK3 is an upstream activator of p38$\alpha$, p38$\beta$, and p38$\gamma$, but not p38$\beta$ (48), and that p38$\gamma$ is not expressed in kidney (49), we postulate that p38$\alpha$ and p38$\beta$ may be the major isoforms involved in regulating EGF receptor activation under this condition. In support of this hypothesis, overexpression of dominant negative of p38$\alpha$ completely blocked p38 phosphorylation but only partially inhibited EGF receptor phosphorylation induced by oxidant injury. The specific role of p38$\alpha$ in regulating EGF receptor activation needs further investigation.

The mechanism underlying p38 activation of the EGF receptor remains unclear. It has been proposed that ligand-dependent and -independent mechanisms are involved in the transactivation of the EGF receptor (50). Ligand-independent activation of the EGF receptor appears to be mediated by multiple intracellular signaling molecules including Src. Src is a non-receptor tyrosine kinase (30, 45) and has been reported to directly activate the EGF receptor by phosphorylation of the EGF receptor at tyrosine residue 845 (51). In this regard, our previous studies have shown that EGF receptor activation needs further investigation.

An alternative mechanism for EGF receptor activation by p38 may involve metalloprotease-dependent EGF receptor ligand production from membrane-bound precursors. Heparin-binding EGF-like growth factor, epiregulin, and transforming growth factor $\alpha$ can activate the EGF receptor (52–55). These EGF receptor ligands are synthesized as transmembrane proteins (proforms) and cleaved to yield soluble forms, thereby binding to the EGF receptor and leading to EGF receptor activation. Ectodomain shedding, the proteolytic processing of the extracellular domain of these transmembrane proteins to form soluble factors, is mediated by metalloproteases (56). p38 has been reported to mediate expression and activation of a number of metalloproteases necessary for cleavage of heparin-binding EGF-like growth factor and transforming growth factor $\alpha$ such as matrix metalloprotease-9 and tumor necrosis factor $\alpha$-converting enzyme (57–59). Heparin-binding EGF-like growth factor has been identified in RPTCs and implicated in EGF receptor activation in vascular smooth muscle cells in response to oxidant stress (60). Whereas these observations raise the possibility that heparin-binding EGF-like growth factor cleavage is involved in p38-mediated EGF receptor activation in RPTCs after oxidant injury, our previous studies showed that blocking matrix metalloprotease had no effect on role of the EGF receptor in RPTC dedifferentiation after oxidant injury and the mechanisms of oxidant injury-induced EGF receptor phosphorylation. Our data revealed that after oxidant injury, surviving RPTCs acquired a dedifferentiated phenotype (e.g., elongated morphology and expression of vimentin) and that EGF receptor and p38 activation are required for RPTC dedifferentiation. Furthermore, oxidant injury activated p38, which, in turn, transactivated the EGF receptor. These are the first data that demonstrate that p38-mediated activation of the EGF receptor is essential for the regulation of RPTC dedifferentiation after oxidant injury.

**FIG. 9. Effects of SB203580, PP1, and overexpression of dominant negative p38 or MKK3 on oxidant injury-induced $\beta$-catenin phosphorylation.** Confluent RPTCs were pretreated with 20 $\mu$M SB203580 (A) or 10 $\mu$M PP1 (D) for 1 h or transfected with adenovirus (MOI = 100 pfu) encoding dominant negative p38 (Ad-p38$\alpha$dn) (B), dominant negative MKK3 (Ad-MKK3$\alpha$dn) (C), or LacZ (Ad-LacZ) for 48 h and then exposed to 1 mM H$_2$O$_2$ for 10 min. Cell lysates were immunoprecipitated with anti-$\beta$-catenin antibody and then analyzed by immunoblotting with antibodies to phosphotyrosine and $\beta$-catenin. Representative blots are shown.
EGF receptor activation after a 10-min exposure of RPTCs to H₂O₂ (43).

Takahashi et al. (29) reported that the EGF receptor bound to β-catenin and induced tyrosine phosphorylation of β-catenin in normal human breast epithelial cells in response to ligand stimulation. Our data revealed that EGF receptor activity is required for tyrosine phosphorylation of β-catenin after oxidant exposure. Consistent with the observation that EGF receptor activation is transactivated by a p38-dependent mechanism, H₂O₂-induced β-catenin tyrosine phosphorylation also was inhibited by inactivation of the upstream activators of the EGF receptor p38, MKK3, and Src. Phosphorylation of β-catenin induces the release of β-catenin from the E-cadherin complex, increasing the free cytoplasmic pool of β-catenin and the subsequent relocation of β-catenin to the nucleus (46, 61). Nuclear β-catenin has been associated with enhanced transcription of target genes that are involved in epithelial-mesenchymal transition (30, 62). Activation of the EGF receptor induces cell scattering and a fibroblast-like morphology associated with β-catenin tyrosine phosphorylation (30, 63). Consequently, p38-mediated EGF activation may regulate RPTC vimentin expression and dedifferentiation via a mechanism involving tyrosine phosphorylation of β-catenin. In support of this idea, a recent study suggested that β-catenin transcriptional activity is required for vimentin expression (34).

In summary, our study demonstrates that after oxidant injury, p38 is activated, which in turn stimulates activation of the EGF receptor and tyrosine phosphorylation of β-catenin in RPTCs. Furthermore, p38 is activated by the sequential activation of Src and MKK3. We also provide evidence that p38-mediated EGF receptor transactivation is required for RPTC dedifferentiation. These data, together with the importance of the EGF receptor in mediating RPTC proliferation and migration in vitro and in renal functional recovery after injury in an animal model, suggest that EGF receptor transactivation may be a key molecular event that initiates regenerative responses after renal injury.

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