Reduction of oxidative stress during recovery accelerates normalization of primary cilia length that is altered after ischemic injury in murine kidneys

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Kim JI, Kim J, Jang HS, Noh MR, Lipschutz JH, Park KM. Reduction of oxidative stress during recovery accelerates normalization of primary cilia length that is altered after ischemic injury in murine kidneys. Am J Physiol Renal Physiol 304: F1283–F1294, 2013. First published March 20, 2013; doi:10.1152/ajprenal.00427.2012.—The primary cilium is a microtubule-based nonmotile organelle that extends from the surface of cells, including renal tubular cells. Here, we investigated the alteration of primary cilium length during epithelial cell injury and repair, following ischemia/reperfusion (I/R) insult, and the role of reactive oxygen species in this alteration. Thirty minutes of bilateral renal ischemia induced severe renal tubular cell damage and an increase of plasma creatinine (Pcr) concentration. Compared with the primary cilium length in normal kidney tubule cells, the length was shortened 4 h and 1 day following ischemia, increased over normal 8 days after ischemia, and then returned to near normal 16 days following ischemia. In the urine of I/R-subjected mice, acetylated tubulin was detected. The cilium length of proliferating cells was shorter than that in nonproliferating cells. Mature cells had shorter cilia than differentiating cells. Treatment with Mn(III) tetrakis(1-methyl-4-pyridyl) porphyrin (MnTMPyP), an antioxidant, during the recovery of damaged kidneys accelerated normalization of cilium length concomitant with a decrease of oxidative stress and morphological recovery in the kidney. In the Madin-Darby canine kidney (MDCK) cells, H2O2 treatment caused released ciliary fragment into medium, and MnTMPyP inhibited the deciliation. The ERK inhibitor U0126 inhibited elongation of cilia in normal and MDCK cells recovering from H2O2 stress. Taken together, our results suggest that primary cilium length reflects cell proliferation and the length of primary cilium is regulated, at least, in part, by reactive oxygen species through ERK.

primary cilium; reactive oxygen species; ERK; ischemia; ROS

THE PRIMARY CILIA IS A MICROTUMLLE-BASED NONMOTILE ORGANELLE THAT EXTENDS FROM THE SURFACE OF MAMMALIAN CELLS (33). IT PERFORMS DIVERSE BIOLOGICAL ROLES INCLUDING CHEMOSENSATION, MECHANOSENSATION, AND PHOTOSENSATION (2). IN THE KIDNEY, A SINGLE PRIMARY CILIA IS FOUND ON THE APICAL SURFACE OF ALL RENAL TUBULAR EPITHELIAL CELLS, WITH THE EXCEPTION OF INTERCALATED CELLS (6, 32). THE PRIMARY CILIA ACTS AS A MECHANOSSENSORY ORGANELLE TO DETECT FLUID FLOW AND COMPOSITION AND TO MAINTAIN THE ARCHITECTURE OF THE NEPHRON (20, 21). PRIMARY CILIA ARE ASSOCIATED WITH PROLIFERATION AND DIFFERENTIATION OF KIDNEY EPITHELIAL CELLS UNDER VARIOUS PATHOPHYSIOLOGICAL CONDITIONS, INCLUDING POLYCYSTIC KIDNEY DISEASE (18, 19, 34). RECENTLY, IT WAS SHOWN THAT ISCHEMIA AND REPERFUSION (I/R) INJURY IN KIDNEYS ALTER

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Materials and Methods

Animal preparation. Experiments were conducted with 8-wk-old C57B/6 male mice. Mice were permitted free access to water and standard mouse chow. In all cases, studies were conducted in accordance with the animal experimental procedures approved by the Kyungpook National University Institution Animal Care and Use Committee. The animals were anesthetized with pentobarbital sodium (60 mg/kg body wt ip; Sigma, St. Louis, MO) before I/R or sham surgery. Kidney ischemia was performed by the occlusion of renal pedicles using nontraumatic microaneurysm clamps (Roboz, Rockville, MD) for 30 min as described previously (15). Cohorts of mice were administered manganese(III) tetrakis (1-methyl-4-pyridyl) porphyrin (MnTMPyP); a cell-permeable SOD mimetic; 5 mg/kg body wt; Calbiochem, San Diego, CA) and 0.9% saline (vehicle) intraperitoneally on a daily basis beginning 48 h after ischemia until 24 h before...
harvest. Some of these mice also had injections of 5′-bromo-2′-deoxuryridine (BrdU; 50 mg/kg body wt; Sigma) administered intra-peritoneally 20 h before kidney harvest.

Cell culture. Fifteen to twenty-five passages of Madin-Darby canine kidney cells (MDCK, American Type Culture Collection, Manassas, VA) cultured in MEM with 5% FBS (Mediatech, Manassas, VA) with 100 μ/ml streptomycin/penicillin (S/P) (WelGENE, Daegu, Korea) were used. For immunofluorescence assay of primary cilia, cells were cultured on the coverslip. After confluency, cells were treated with hydrogen peroxide (Sigma), the ERK inhibitor U0126 (Calbiochem, San Diego, CA), MnTMPyP (Calbiochem), or vehicle for the indicated times and conditions. Cells were fixed with 4% paraformaldehyde and processed for the immunofluorescence or lysed for the Western blot analyses.

Histology. Kidneys were perfused via the left ventricle with 30 ml of PBS for 2 min and then PLP (2% paraformaldehyde, 75 mM l-lysine, and 10 mM sodium metaperiodate; Sigma) solution. After harvest, some of these mice also had injections of 5′-bromo-2′-deoxyuridine (BrdU; 50 mg/kg body wt; Sigma) administered intraperitoneally 20 h before kidney harvest.

Western blot analysis. Western blot analyses were performed as described previously (15). Briefly, renal tissue lysates were separated on 10% SDS-PAGE gels and then transferred to Immobilon membranes (Millipore, Bedford, MA). The membranes were incubated with anti-p-ERK (Cell Signaling, Beverly, MA), anti-total ERK (Cell Signaling, Beverly, MA), anti-AQP1 (anti-AQP1; 1:100 dilution; Alomone Laboratories, Jerusalem, Israel), and anti-AQP2 (1:200 dilution; Alomone Laboratories) antibodies overnight at 4°C. After incubation, the sections were washed three times in PBS for 5 min each, incubated with FITC or Texas Red-conjugated secondary antibodies for 60 min at room temperature, and washed three times with PBS for 5 min each. To detect the cell nuclei, 4′,6-diamidino-2-phenylindole (DAPI; Sigma) was applied to the sections for 1 min each. Finally, the sections were mounted with the Prolong Gold anti-fade reagent (Invitrogen, Carlsbad, CA) and observed under an Axioskop-2 epifluorescence microscope (Carl Zeiss). Each experimental animal group consisted of three mice.

Measurement of primary cilium length. Kidney sections were processed for immunofluorescence microscopy by staining with anti-acetylated α-tubulin antibody and DAPI as described above. Images were captured using a Axioskop-2 epifluorescence microscope (Carl Zeiss). Five to ten fields were randomly captured (×400) and measured in each segment per time point from four independent animals. For measurement of length of primary cilia in MDCK cells cultured on the coverslips, five different fields were randomly captured from three independent experiments. More than 50 cells from each experiment were used to measure length of cilia. iSolution (IMT i-Solution, Rochester, NY) software was used to trace and measure the length of cilia in captured images.

Measurements of superoxide formation and lipid peroxidation in kidney tissue. Superoxide levels in kidney tissue were measured using dihydroethidium (Sigma; Ref. 13) in a fluorescence spectrometer (Molecular Devices, Sunnyvale, CA). In brief, kidneys excised from the mice were immediately homogenized on ice with a Dounce homogenizer. Then, 200 μl of 10 μM dihydroethidium were added to 96-well plates containing 20 μl of kidney lysates. Plates were read using an emission/excitation filter of 530/620 nm at a temperature of 37°C. Superoxide levels were expressed as the value per milligram protein of kidney lysates. Thiobarbituric acid-reactive substances (Sigma-Aldrich were used to measure lipid peroxidation; Ref. 12).

Renal functional parameter. Concentration of plasma creatinine (PCr) was measured using a Beckman Creatinine Analyzer II (Beckman, Brea, CA).

Immuno-fluorescence. Paraffin-embedded tissue sections were deparaffinized with xylene; rehydrated with 100, 95, and 80% ethanol; and then washed with PBS for 10 min each. The sections were incubated in PBS containing 0.1% sodium dodecyl sulfate (SDS; Sigma) for 5 min and washed for 10 min in PBS. To reveal the antigen epitope, the sections were boiled in 10 mM sodium citrate buffer (pH 6.0) for 10 min using an autoclave, cooled for 20 min at room temperature, and then washed three times with PBS for 5 min each. The sections were blocked with PBS containing 1% bovine serum albumin (blocking buffer) for 30 min at room temperature and then incubated with anti-acetylated-tubulin (1:100; Sigma-Aldrich), anti-Na/K-ATPase (1:20 dilution; Santa Cruz, Santa Cruz, CA), anti-BrdU (1:100 dilution; Serotec, Raleigh, NC), anti-aquaporin-1 (anti-AQP1; 1:100 dilution; Alomone Laboratories, Jerusalem, Israel), and anti-AQP2 (1:200 dilution; Alomone Laboratories) antibodies overnight at 4°C. After incubation, the sections were washed three times in PBS for 5 min each, incubated with FITC or Texas Red-conjugated secondary antibodies for 60 min at room temperature, and washed three times with PBS for 5 min each. To detect the cell nuclei, 4′,6-diamidino-2-phenylindole (DAPI; Sigma) was applied to the sections for 1 min each. Finally, the sections were mounted with the Prolong Gold anti-fade reagent (Invitrogen, Carlsbad, CA) and observed under an Axioskop-2 epifluorescence microscope (Carl Zeiss). Each experimental animal group consisted of three mice.

Fig. 1. Kidney morphology and concentration of plasma creatinine (PCr) following ischemia and reperfusion (I/R) injury. Mice were subjected to 30 min of bilateral renal ischemia or a sham operation on day 0. A: kidney sections were periodic acid-Schiff stained. B: PCr concentration was determined at the indicated times (n = 6 per time point). Results are expressed as means ± SE. *P < 0.001 vs. respective day 0 (before surgery).
Fig. 2. Alterations of primary cilium length in kidney cells and release of primary cilia into the urine after ischemia and reperfusion. Mice were subjected to 30 min of bilateral renal ischemia. Kidneys were harvested before and 1, 8, and 16 days after ischemia. Kidney sections were double-stained with anti-acetylated tubulin (A, C, E, G, and I), and anti-Na\(^+\)-K\(^+\)-ATPase (C and G), anti-aquaporin (AQP)-1 (E), or anti-AQP-2 (I) antibodies. 4'-6-Diamidino-2-phenylindole (DAPI; blue) indicates nuclear staining (A, C, E, G, and I). B: lengths of 30 primary cilia per kidney (n = 3) were measured and averaged. Results are expressed as means ± SE. Arrow indicates primary cilium. C and D: pictures were taken from the cortex and outer medulla. Na/K-ATPase (green) was more strongly expressed in the distal tubules than the proximal tubules. One day after ischemia, cilia on tubule cells in the outer medulla were disrupted more severely than those in the cortex. Primary cilia (green) were observed in the lumen and the cilia lengths changed variably depending on the region. Cilia length changes were more dramatic in the outer medulla than the cortex. E and F: images were taken from the outer medulla and the inner medulla. Lengths of primary cilia of AQP-1-positive (red) descending thin limb of Henle cells, following ischemia, did not change depending on the location in the inner or outer medulla. Cilia length was normal at 1 day following ischemia, increased at 8 days following ischemia, and was normal again at 16 days following ischemia. G and H: images of distal tubule cells were taken from the cortex and outer medulla. Na-K-ATPase (green) was strongly expressed in the distal tubule cells. Primary cilia (green) were observed in the lumen. Cilia length was normal at 1 day following ischemia, increased at 8 days following ischemia, and normal again at 16 days following ischemia. I and J: images of collecting duct cells were taken from the inner and outer medulla and cortex. AQP-2 (red) was used as a maker for collecting duct cells. Length of primary cilia in the collecting duct cells changed depending on their location and postischemic times. Cilia length in collecting duct cells was decreased in the cortex and outer medulla at 1 day following ischemia, increased in the cortex, outer medulla and inner medulla at 8 days following ischemia, and normal in the cortex, outer medulla and inner medulla at 16 days following ischemia. K: urines were collected at 4 and 24 h after ischemia and used for Western blot analysis using anti-acetylated tubulin (Ac-tubulin), anti-ARL13B, and anti-GAPDH antibodies. *P < 0.05 vs. day 0; #P < 0.05 vs. other groups at day 1; $P < 0.05 vs. other groups at day 8 or 16.
Fig. 2—Continued
indicates that histological restoration of I/R-injured kidneys was not complete (Fig. 1B).

Altersations of primary cilia lengths after ischemia. Primary cilia were found on renal tubular epithelial and parietal cells, but not, as previously reported (7), on intercalated cells of the collecting ducts (Fig. 2). The length of primary cilia in cells of the parietal layer of the Bowman’s capsule were shorter at 1 day, longer at 8 days, and similar at 16 days following I/R injury, when compared with sham controls (Fig. 2, A and B).

When primary cilia length was determined in proximal tubule cells at 1 day following I/R injury, the length of cilia on cells in the outer medulla, but not in the cortex, was shorter when compared with sham-operated control cells. This could indicate that the primary cilia in the proximal tubular epithelial cells in the outer medulla were more susceptible to I/R injury than those in the cortex (Fig. 2C). Eight days following ischemia the lengths of primary cilia in the proximal tubule cells both in the cortex and outer medulla were significantly longer compared with those in the sham-operated kidneys (Fig. 2, C and D). Sixteen days after I/R the length of primary cilia in the proximal tubular epithelial cells in the cortex, but not in the outer medulla, returned to near normal ranges (Fig. 2, C and D).

The length of primary cilia in the cells of descending thin limbs in the inner and outer medulla was not changed 1 day after ischemia, but the length was significantly longer 8 days after ischemia, and then returned to normal level 16 days after ischemia (Fig. 2, E and F). The length of primary cilia in the distal tubule cells in the cortex and outer medulla 1 day after ischemia was not different compared with that of normal controls; however, 8 days after ischemia the length of the primary cilia in the distal tubule cells was longer than in normal controls. The length of primary cilia 16 days after ischemia was not different compared with that of normal controls; however, 8 days after ischemia the length of the primary cilia in the distal tubule cells was longer than in normal controls. The length of primary cilia 16 days after ischemia was not different compared with that of normal controls; however, 8 days after ischemia the length of the primary cilia in the distal tubule cells was longer than in normal controls.

Functional and histological changes in the kidney after I/R injury. PCr dramatically increased 24 h after ischemia and then returned to normal levels at 8 and 16 days later (Fig. 1A). Concurrent with the increase in PCr concentration, disruption, dilation, flattening, and congestion of tubules appeared in the kidney at 24 h after ischemia (Fig. 1B). Damage was most severe at the proximal tubules in the outer medulla, compared with other regions (Fig. 1B). Eight and sixteen days after ischemia the number of atrophic and dilated tubules was much less than that seen at 24 h following ischemia (Fig. 1B). This indicates that histological restoration of I/R-injured kidneys occurred; however, even after 16 days following the I/R injury, histological recovery was not complete (Fig. 1B).

Detection of primary ciliary fragment in the urine of the mouse. Urine was collected from sham- or I/R-subjected mouse using metabolic cage for the indicated time. To make the Western blot sample, SDS sample buffer was added to urine and then boiled for 5 min at 98°C. Western blot analyses were performed using anti-acetylated-tubulin antibody as described above.

Detection of primary ciliary fragment in the cell culture medium. Culture medium was collected at indicated time and condition. The medium sample for Western blot was prepared in same manner with urine sample.

Statistics. The results were expressed as the means ± SE. Statistical differences among groups were calculated via ANOVA followed by a least significant difference post hoc comparison using the SPSS 12.0 program. Differences between groups were considered statistically significant at a P value of < 0.05.

RESULTS

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ischemia was no longer different from that of normal controls (Fig. 2, G and H). The primary cilia length in collecting duct cells in the cortex and outer medulla 24 h after ischemia was significantly decreased compared with normal controls, whereas the length of cilia of the cells in the inner medulla was not different from that of normal kidney (Fig. 2, I and J). Eight days after ischemia the length of the primary cilia in collecting duct cells from all parts of the kidney was longer than that in normal kidney, and the length of the collecting cells in the outer medulla was longest. Sixteen days after ischemia the length of the primary cilia in collecting duct cells from all regions of the kidney returned to normal (Fig. 2, I and J).

To test that the shortening of primary cilia is caused by deciliation, we determined ciliary fragment in the urine after sham or I/R by immunoblotting using anti-acetylated tubulin antibody. Anti-acetylated tubulin was detected from 4 h after ischemia and increased at 24 h after ischemia. Anti-acetylated tubulin was not detected in sham-operated control (Fig. 2K), indicating shortening of cilia occurred, at least, in part, by deciliation. ADP-ribosylation factor-like protein 13B (ARL13B), which is located in the primary cilia and plays a role in cilia formation (3), was detected 24 h, but not 4 h, urine sample (Fig. 2K), indicating that primary cilia extruded to cell surface are more sensitive to I/R injury than basal body. GAPDH protein was not detected urine samples (Fig. 2K).

Length of primary cilia in proliferating cells. To investigate if the primary cilia is associated with cell proliferation, we determined the primary cilia length in regenerating tubular epithelial cells. To identify regenerating cells, BrdU was injected into mice for 20 h before the kidneys were harvested. BrdU incorporation reveals proliferating cells due to incorporation of BrdU into DNA during S phase of the cell cycle (7). BrdU-positive cells had very short primary cilia or no primary cilia (Fig. 3, A and B).

Acceleration of normalization of primary cilia length by MnTMPyP treatment. To investigate an involvement of ROS in changes of primary cilia length after I/R injury, we administered six daily treatments of MnTMPyP, a superoxide scavenger. The MnTMPyP treatments for the mice subjected to I/R injury were started 2 days following ischemia. We then determined levels of superoxide formation and the length of primary cilia. The length of primary cilia in the tubular epithelial cells in the outer medulla of MnTMPyP-treated mice was decreased compared with those in vehicle-treated control mice (Fig. 4, A–C). Consistent with the previous study (18), kidney morphology in the MnTMPyP-treated I/R mice was improved compared with the vehicle-treated mice (data not shown). This suggests that antioxidant treatment accelerates the restoration of primary cilia length that was altered by I/R. Superoxide formation in MnTMPyP-administered mouse kidneys was lower than that in vehicle-administered, control, kidneys, confirming the antioxidant effect of MnTMPyP (Fig. 5).

Next, we investigated ERK activation, because ROS activate ERK (8, 16) and ERK activation is involved in renal injury and repair following I/R (15, 17). In addition, ERK is known to increase cilia length (1, 31). Phosphorylated ERK and total levels 8 days after ischemia were increased and the increases were significantly inhibited by MnTMPyP treatment (Fig. 6).

Inhibition of deciliation of MDCK cells by MnTMPyP treatment. To test that deciliation of kidney tubule cells are induced by oxidative stress, we examined that hydrogen peroxide (H2O2) treatment induces deciliation of MDCK cells. The 1-mM H2O2 treatment for 4 h increased anti-acetylated tubulin release from cells into culture medium (Fig. 7A). Pretreatment of MnTMPyP prevented the H2O2-induced deciliation, indicating deciliation by oxidative stress. We also confirmed these results by staining of cilia in the fixed MDCK cells with H2O2 treatment with/without pretreatment of MnTMPyP (Fig. 7B).

Inhibition of elongation of primary cilia of MDCK cells by specific ERK inhibitor treatment. To test that activation of ERK involves in elongation of cilia, we determined ciliary length in vehicle-treated or U0126-treated, an inhibitor of ERK activation, MDCK cells. Treatment of U0126 clearly inhibited elongation of cilia compared with nontreatment (Fig. 8). Shortened length of primary cilia after H2O2 treatment was lengthened over time (Fig. 9A). This lengthening of primary cilia was inhibited by U0126 treatment (Fig. 9B).
DISCUSSION

The primary cilium has been long recognized as a mechanosensory organelle, and recent data have demonstrated that its length is associated with I/R injury to the kidney (28, 29). However, much remains to be studied about changes in primary cilium length postischemia, both with respect to the different kidney cell types and chemical regulators of cilia length. In this study, we report that post-I/R alteration of primary cilia length differs depending on the tubular segment and time course following I/R. In addition, we report for the first time that ROS regulates primary cilia length.

In general, kidney tissues exposed to transient ischemia undergo serial changes: injury very early after ischemia, proliferation several days following ischemia, and differentiation or transdifferentiation occurring from several days to weeks depending on the severity of injury (5, 12, 14). Overgaard et al. (13a) reported that remodeling of epithelium injured by stress is associated with reversible deciliation. In this present study the primary cilia length is differentially changed depending on the tubular segment and times following I/R. For example, 1 day after ischemia, the lengths of primary cilia were shortened in the proximal tubular epithelial cells in the outer medulla but...
Fig. 4—Continued
not in cortex. Furthermore, the lengths of primary cilia in the
distal tubular epithelial cells in the cortex and outer medulla
were not changed, whereas the lengths of primary cilia in
collecting ducts in the cortex and outer medulla were short-
ened. These data indicate that I/R injury caused variable
shortening of primary cilia length depending on tubule segment
and location, suggesting that primary cilia length may be a
marker of cell injury.

Shortening of primary cilia length occurs by two distinct
mechanisms; reabsorption and deciliation (22, 26). Reabso-
ption is a normal process in which cells gradually retract the
cilium. Primary cilia present in G1/G0 cells and ciliary reab-
sorption occur as the cell cycle progresses to S phase of cell
cycle. Complete ciliary reabsorption is necessary to release the
centriole basal body before mitosis (27). Loss of primary cilia
is required for the development of epithelium in multiple
organs including kidneys (23, 25). Tubular cell regeneration
peaks at 3 days after I/R in the proximal tubule of the outer
medulla (9, 11). Therefore, the shortening of primary cilia that
occurred in BrdU-positive cells at 3 days following I/R injury
may indicate a reabsorption of primary cilia in proliferating
cells. In contrast, deciliation is a rapid shedding of cilia in
response to environmental stress including I/R injury (4, 22).
Therefore, the shortening seen at 1 day after ischemia may
reflect deciliation by stress. Here, we showed that ciliary
fragments are released into urine and cell culture medium after
I/R and oxidative stress, in an vitro model of I/R, respectively.
This indicates that deciliation occurs in response to I/R. Fur-
thermore, attenuation of oxidative stress by the MnSOD mi-
metic MnTMPyP inhibited deciliation in MDCK cells support-
ing the idea.

In the recovery phase, 8 days following ischemia, primary
cilia were elongated compared with normal control cilia in all
nephron segments and collecting ducts regardless of location.
Sixteen days following ischemia, during the differentiation
phase, the lengths of primary cilia in most of nephron segments
returned to normal levels, except in parietal cells and proximal
tubular cells of the outer medulla. The primary cilia lengths in
the proximal tubular epithelial cells of the outer medulla and
parietal epithelial cells were longer than those of controls. The
delay in the recovery of primary cilia length in the proximal
tubule cells may be due to the fact that proximal tubule cells of
the outer medulla tend to be most severely damaged compared
with other cells. In addition, the delay in the recovery in
parietal cells may be caused by the pressure change of the renal

Fig. 5. Effect of MnTMPyP treatment of superoxide formation (A) and lipid peroxidation (B) in the kidneys after ischemia and reperfusion. Mice were subjected to 30 min of bilateral renal ischemia (ischemia) or sham operation (sham) on day 0. Some mice were treated with either MnTMPyP (5 mg/kg body wt) or saline (vehicle) daily, beginning 2 days following the operation. Eight days after the operations, the kidneys were harvested and measurements of superoxide formation and lipid peroxidation were performed. Values are expressed as means ± SE (n = 4). *P < 0.05 vs. sham.

Fig. 6. Effect of MnTMPyP treatment of ERK activation in the kidneys after ischemia and reperfusion. Mice were subjected to 30 min of bilateral renal ischemia on day 0. Some mice were treated with either MnTMPyP (5 mg/kg body wt) or saline (vehicle) daily, beginning 2 days following the operation. Eight days after the operations, the kidneys were harvested and phosphorylated (p)- and total (t)-ERK levels were determined. GAPDH expression was used as a loading control. Densities of blots were quantified using the Lab Works program. Values are expressed as means ± SE (n = 4). * p<0.05 vs. vehicle-treated sham; #, p<0.05 vs. vehicle-treated ischemia.
corpuscle, which stimulates responses of parietal cells. Ureteral obstruction, which increases the pressure of Bowman’s capsule, increased cilia length (30). Verghese and colleagues (30) observed that primary cilia length was increased at 7 days following ischemia and then returned to normal length over a longer time period. In that study, they suggested that the lengthening of primary cilia is associated with the reconstruction of epithelial phenotypes by acceleration of cell-differentiation-related factors (28, 30). Several studies have proposed that lengthening of primary cilia in kidney epithelial cells is important for epithelial differentiation, maintaining the epithelial phenotype, and enhancing compensatory adaptation (23, 25, 30). Taken together, our data demonstrate that the length of primary cilia may be a sensitive marker to monitor kidney cell fates and primary cilia may play important roles in cell proliferation and differentiation to maintain renal function.

Therefore, it is important to determine the mechanisms involved in the regulation of primary cilia length. We investigated if ROS is associated with the alteration of primary cilia length, because ROS is highly associated with injury and repair of tubular epithelial cells (10, 12, 14). Recently, we demonstrated that kidney tissue exposed to I/R have high levels of ROS and that normalization of the increased ROS levels accelerate the repair of tubular epithelial cells and prevent progression to fibrosis (9). In the present study, removal of ROS, which increased after ischemia, accelerated normalization of primary cilia length.

In the present study we found that postischemic kidneys had greater activation of ERK than control kidneys and that antioxidant, MnTMPyP treatment reduced the ERK activation with shortening of primary cilia length. This indicates that increased ERK activation may lengthen primary cilia. Recently, Wann and Knight (31) reported that inflammatory cytokine interleukin-1β increased cilia length in freshly isolated chondrocytes and NIH3T3 fibroblasts via PKA, MEK-ERK, and PKC. Abdul-Majeed et al. (1) reported that MAPK activation elongated primary cillum length. We also recently reported that the overexpression of Sec10, a central
component of the exocyst complex, resulted in increased levels of ERK phosphorylation and longer primary cilium in cultured kidney tubular epithelial cells (16, 35). Therefore, the increase in primary cilia length in post-I/R kidney tubular epithelial cells may be a result of increased ERK activation. Our results that inhibition of ERK in MDCK cells and restoring MDCK cells from oxidative stress injury inhibit elongation of primary cilia support that idea in the present study. Acceleration of normalization of primary cilia length by MnTMPyP may be associated with an inhibitory effect on ERK phosphorylation.

Fig. 9. Effect of ERK activation in the elongation of primary cilium in recovering MDCK cells from oxidative stress. A: MDCK cells grown on the coverslip were treated with 1 mM H$_2$O$_2$ for 4 h and then fixed 1, 2, 3, and 4 days after H$_2$O$_2$ treatment times. Fixed cells were immunofluorescence-stained using anti-acetylated-tubulin antibody (green). B: some cells were treated with either vehicle or 10 µM of U0126 beginning at 1 day after 1 mM H$_2$O$_2$, daily, fixed at 4 days after 1 mM H$_2$O$_2$ treatment, and then immunofluorescence stained using anti-acetylated-tubulin antibody (green). DAPI (blue) indicates nuclear staining (n = 4).

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

REFERENCES


