Aldosterone induces epithelial-mesenchymal transition via ROS of mitochondrial origin

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Zhang A, Jia Z, Guo X, Yang T. Aldosterone induces epithelial-mesenchymal transition via ROS of mitochondrial origin. *Am J Physiol Renal Physiol* 293:F723–F731, 2007. First published June 27, 2007; doi:10.1152/ajprenal.00480.2006.—It has been well appreciated that aldosterone (Aldo) plays a direct profibrotic role in the kidney but the underlying mechanism is unclear. We examined the role of Aldo in epithelial-mesenchymal transition (EMT) both in vitro and in vivo. Exposure of human renal proximal tubular cells to Aldo for 48 h dose dependently induced EMT as evidenced by conversion to the spindle-like morphology, loss of E-cadherin, and de novo expression of α-smooth muscle actin (SMA); the effect was noticeable at 50 nM and maximal at 100 nM. The EMT was completely blocked by the selective mineralocorticoid receptor (MR) antagonist eplerenone. Aldo time dependently increased intracellular reactive oxygen species (ROS) production that was detectable at 15 min and peaked (2.3-fold) at 60 min, as assessed by 2′,7-dichlorofluorescin diacetate fluorescence. Aldo-induced oxidative stress and EMT were both abolished by the mitochondrial respiratory chain complex I inhibitor rotenone, but not the NADPH oxidase inhibitor apocynin. Aldo induced phosphorylation of ERK1/2 that was completely blocked by rotenone. Male 129-C57/BL6 mice were treated with deoxycorticosterone acetate (DOCA) salt (subcutaneous implantation of 50 mg of DOCA pellet plus 1% NaCl as drinking fluid) for 3 wk and animals were treated with vehicle or rotenone (600 ppm in diet) for the last week. DOCA salt induced a 2.5-fold increase in α-SMA and a 30% reduction of E-cadherin, as assessed by real-time RT-PCR, that were both restricted to renal epithelial cells, as determined by immunohistochemistry. In contrast, DOCA salt-induced changes in α-SMA and E-cadherin were completely blocked by treatment with rotenone. These observations suggest that Aldo induces EMT via MR-mediated, mitochondrial-originated, ROS-dependent ERK1/2 activation in renal tubular epithelial cells.

Reagents and antibodies. Aldo, apocynin, rotenone, N-acetyl-L-cysteine (NAC), and mouse monoclonal anti-α-smooth muscle actin (SMA) antibody were purchased from Sigma (St. Louis, MO). Extracellular signal-regulated kinases 1/2 (ERK 1/2) inhibitor, U0126, was purchased from Calbiochem (Cambridge, MA). Eplerenone, a specific MR blocker, was provided by Pfizer (New York, NY). Mouse monoclonal anti-E-cadherin antibody was provided from BD PharMingen (San Diego, CA). Rabbit monoclonal antiphospho-p44/42 ERK 1/2 (Thr202/Tyr204) and anti-total p44/42 ERK1/2 antibodies were purchased from Cell Signaling Technology (Beverly, MA). Rabbit anti-MR polyclonal antibody, fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG, horseradish peroxidase-conjugated goat anti-mouse and anti-rabbit IgG were from Santa Cruz Biotechnology (Santa Cruz, CA).

Mice and experimental protocol. Eight-week-old male 129/Sv-C57BL/6J mice (25–30 g body wt) were implanted subcutaneously with 21-day-release DOCA pellets containing 50 mg DOCA (Innovative Research of America, Sarasota, FL) by incision of the right flank region under light 3% isoflurane anesthesia. After 14 days, DOCA-treated animals received vehicle or rotenone (600 ppm in diet) for 7 days. All animals (control, DOCA salt, and DOCA salt plus rotenone) received 1% NaCl in tap water starting on the first day of DOCA treatment. All mice were placed on standard pelleted rodent chow and housed in an air-conditioned room with a 12:12-h light-dark cycle. All animal proce-
dures were approved by the University of Utah Institutional Animal Care and Use Committee.

**Human kidney-2 cells.** HK-2 cells, the immortalized human proximal tubular cell line, were grown in keratinocyte serum-free media (KSFM) supplemented with bovine pituitary extract and epidermal growth factor (Invitrogen). The cells were grown at 37°C in a humidified 5% CO2 incubator and subcultured at 50–80% confluence using 0.05% trypsin-0.02% EDTA (Invitrogen).

**RT-PCR and real-time RT-PCR.** Total RNA was isolated from HK-2 cells or whole kidney tissues using TRIzol Total RNA Isolation kit (Invitrogen) according to the manufacturer’s protocol. The RNA was eluted with RNase-free water. Reverse transcription was performed using the Superscript III RT kit (Invitrogen) according to the manufacturer’s protocols. Briefly, the reactions were incubated at 65°C for 5 min and then at 50°C for 60 min. Oligonucleotides (Table 1) were designed by Primer3 software (available at http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and synthesized at the University of Utah. Real-time PCR amplification was performed using the SYBR Green master mix (Applied Biosystems) and the Prism 7500 Real-time PCR Detection System (Applied Biosystems). Cycling conditions were 95°C for 10 min followed by 40 repeats of 95°C for 15 s and 60°C for 1 min. Relative amounts of mRNA were normalized by β-actin or GAPDH and calculated using the delta-delta method from threshold cycle numbers (35).

**Fluorescent immunocytochemistry.** Cells were grown on coverslips and stimulated with Aldo (100 nM). The medium was removed, and the cell layer was rinsed with PBS. Cells were fixed and permeabilized with acetone-methanol for 10 min at 20°C and then were rehydrated with PBS and blocked with 5% BSA in PBS for 1 h. Coverslips were sequentially incubated with rabbit monoclonal anti-MR, mouse monoclonal anti–E-cadherin, mouse monoclonal anti–smooth muscle actin (SMA), or the appropriate secondary antibody. Cells were then incubated with DAPI, and the slides were mounted with Vectashield mounting medium.

**Fig. 1.** Aldosterone (Aldo)-induced epithelial-mesenchymal transition (EMT). A and B: morphological changes. The cells were grown in 6-well plates until 80% confluence and then treated with vehicle (A) and Aldo (100 nM; B) for 48 h. Photographs were taken using a Nikon microscope (phase contrast). C and D: real-time RT-PCR analysis for E-cadherin and α-smooth muscle actin (SMA) expression. The cells were treated with Aldo (10–100 nM) for 12 h and E-cadherin (C) and β-SMA (D) expression was detected by real-time RT-PCR. E and F: Western blotting analysis for E-cadherin and α-SMA. The cells were treated with Aldo (10–100 nM) for 48 h and E-cadherin (E) and α-SMA (F) expression was detected by immunoblotting. Top: representative immunoblots. Bottom: densitometric analysis; n = 3 for each group. *P < 0.01 vs. control (P < 0.01, 1-way ANOVA for C, D, E, and F).

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**Table 1. Real-time PCR primers used in the study**

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**F724**  
**ALDOSTERONE INDUCES EMT**
clonal anti-α-SMA, or E-cadherin and FITC-labeled goat anti-rabbit or anti-mouse antibodies each for 60 min at room temperature. Cells were then visualized and photographed by fluorescence microscopy at ×200 or ×400 magnification. Negative controls were performed using nonimmune serum or IgG instead of first antibodies.

**Fluorescent immunohistochemistry.** The kidneys were fixed in 4% paraformaldehyde and imbedded with paraffin. Paraffin sections of each specimen were cut at 5 μm (Cryostat 2800 Frigocut-E, Leica Instruments) and a standard protocol using xylene and graded ethanol was employed to deparaffinize and rehydrate. The sections were washed with K200 or H11003 were then visualized and photographed by fluorescence microscopy at 37°C for permeabilization. The sections were then incubated overnight at 4°C with mouse monoclonal anti-E-cadherin (A, C) or Aldo (100 nM; B, D) for 48 h. Immunofluorescence was performed using mouse monoclonal anti-E-cadherin (A, B) or anti-α-SMA (C, D) and FITC-labeled goat anti-mouse and FITC-labeled goat anti-rabbit clonal anti-ERK1/2 antibodies. Cells were visualized and photographed by fluorescence microscopy at ×200 magnification for E-cadherin and ×400 magnification for α-SMA.

**DCFDA fluorescence measurement of reactive oxygen species.** The fluorogenic substrate 2′,7′-dichlorofluorescein diacetate (DCFDA) is a cell-permeable dye that is oxidized to highly fluorescent 2′,7′-dichlorofluorescein (DCF) by H2O2 and can therefore be used to monitor intracellular generation of reactive oxygen species (ROS). For measurement of ROS, cells were grown on glass coverslides. When the cells reached confluence, they were washed twice with PBS and incubated for 30 min with 10 μM DCFDA and then treated with Aldo in the presence or absence of apocynin or rotenone at appropriate concentrations. At the end of the incubation period, the cells were again washed twice with PBS and imaged by fluorescence microscopy.

**Western blotting.** HK-2 cells were lysed and subsequently sonicated in PBS containing 1% Triton X-100, 250 μM phenylmethane-sulfonfyl fluoride (PMSF), 2 mM EDTA, and 5 mM diithiothreitol (DTT; pH 7.5). Protein concentrations were determined by Coomassie reagent. Thirty micrograms of protein from whole cell lysates were denatured in boiling water for 10 min, separated by SDS-PAGE gel, and transferred onto nitrocellulose membranes. The blots were blocked overnight with 5% nonfat dry milk for 1 h and incubated overnight at 4°C with the primary antibodies against phospho-ERK1/2 at a dilution of 1:1,000. After being washed with TBS, blots were incubated with a goat anti-horseradish peroxidase-conjugated secondary antibody and visualized with ECL kits (Amersham).

**Phosphorylation of MAP kinases.** HK-2 cells grown in a six-well plate were lysed by sonication for 10 s in 200 μl of 1× Laemmli sample buffer containing 10 mM Tris, 1.4% SDS, and 40 mM DTT (pH 6.8). The protein samples were heated at 60°C for 15 min and electrophoresis was performed as described above. The blots were blocked in 5% nonfat dry milk for 1 h and incubated overnight at 4°C with the primary antibodies against phospho-ERK1/2 at a dilution of 1:1,000. The secondary antibody and ECL reaction were the same as described above.

**Fig. 2.** Fluorescent immunocytochemistry for E-cadherin and α-SMA. The cells were grown on the coverslides until 80% confluence and then treated with vehicle (A, C) or Aldo (100 nM; B, D) for 48 h. Immunofluorescence was performed using mouse monoclonal anti-E-cadherin (A, B) or anti-α-SMA (C, D) and FITC-labeled goat anti-mouse antibodies. Cells were visualized and photographed by fluorescence microscopy at ×200 magnification for E-cadherin and ×400 magnification for α-SMA.

**Fig. 3.** Expression of mineralocorticoid receptor (MR) in HK-2 cells. A: RT-PCR analysis of the MR was performed from RNA isolated from HK-2 cells. Ethidium bromide-stained agarose gels for the MR. B: immunoblotting of the MR from HK-2 cells. C: fluorescent immunocytochemistry of the MR in HK-2 cells. HK-2 cells were incubated in keratinocyte serum-free media (KSF M) in the absence (left) and presence (right) of Aldo for 60 min and then were fixed with acetone-methanol and stained with MR antibody. Magnification ×400.
microscopy. To quantitate ROS levels, cells were seeded to a 96-well plate and treated as described above. Relative fluorescence was measured using a fluorescence plate reader (FLUOstar OPTIMA) at excitation and emission wavelengths of 485 and 528 nm, respectively, three times at 90-s intervals.

Statistical analysis. Values shown represent means ± SE. Statistical analysis was performed by one-way ANOVA and Bonferroni tests with a P value of <0.05 being considered statistically significant.

RESULTS

Aldo-induced EMT. To evaluate EMT, we used three independent parameters: cell morphology, the expression of E-cadherin, and α-SMA. The morphological changes were assessed by phase contrast microscopy. Control HK-2 cells formed a confluent monolayer and exhibited cobblestone-like morphology (Fig. 1A); while following exposure to 100 nM Aldo for 48 h, the cells exhibited striking morphological changes, displaying an elongated and fibroblast-like morphology (Fig. 1B).

E-cadherin is the most commonly expressed cadherin in epithelial cells and is widely used as an epithelial marker (29, 34). We monitored the changes in E-cadherin mRNA and protein expression over the 48-h period of Aldo treatment. This treatment dose dependently reduced E-cadherin mRNA and protein expression with a noticeable effect at 50 nM and a maximal effect at 100 nM (Fig. 1, C and E). This result was confirmed by immunofluorescence showing abundant expression of E-cadherin on the basolateral membrane in the basal state (Fig. 2A) and a remarkable reduction of the expression following Aldo treatment (Fig. 2B).

α-SMA is a phenotypic marker of myofibroblast cells and its expression is a feature of advanced stages of EMT (16). A 48-h Aldo treatment dose dependently induced α-SMA mRNA expression (Fig. 1D). This was paralleled with the increases in α-SMA protein expression (Fig. 1F). Furthermore, the induction of α-SMA stress fiber formation following Aldo treatment was visualized by immunofluorescence.
cent microscopy (Fig. 2D) contrasting with the nearly negative staining in untreated cells (Fig. 2C).

Role of MR in Aldo-induced EMT. To address this issue, we first examined whether MR was expressed in cultured HK-2 cells. RT-PCR and immunoblotting revealed the presence of both MR mRNA and protein in these cells (Fig. 3, A and B). Immunofluorescence showed staining of MR antibody almost exclusively in the cytosol in basal state (Fig. 3C) but in the nucleus following 60 min of Aldo treatment, indicating nearly complete translocation of MR (Fig. 3D). To address the functional role of MR, we examined the effect of the MR antagonist eplerenone, the EMT was almost completely prevented as assessed by changes in morphology, α-SMA, and E-cadherin.

Aldo-induced ROS production and its origin. Given the recent evidence for involvement of ROS in the EMT induced by TGF-β1 (22) and MMP-3 (19), we tested the possibility that ROS might mediate EMT in our experimental model. DCF fluorescence indicating ROS production was significantly intensified following 60 min of Aldo treatment as visualized by fluorescence microscopy (Fig. 5, A and B). ROS levels were further quantified using a fluorescence microplate reader. As shown in Fig. 5C, Aldo time dependently induced ROS production that was noticeable at 15 min and maximal at 60 min (2.3-fold).

Subsequent experiments were undertaken to determine the source of Aldo-induced ROS production, e.g., NADPH oxidase vs. mitochondria. This was archived by the use of the NADPH oxidase inhibitor apocynin and the mitochondrial respiratory chain complex I inhibitor rotenone. The induction of ROS production in response to Aldo treatment was completely blocked by rotenone but was unaffected by apocynin (Fig. 5D). In line with this finding, Aldo treatment at the same dose range (10−100 nM) capable of elevating ROS production did not affect gene expression of either gp91phox or p47phox, two major subunits of NADPH oxidase (Fig. 5, E and F).

Activation of ERK1/2 by mitochondrial-derived ROS. Given the potential role of ERK1/2 in mediating TGF-β1-induced EMT (22, 33), we examined whether ERK1/2 served as a target of ROS in Aldo-induced signaling pathway in cultured HK-2 cells. Within minutes, Aldo treatment significantly increased phosphorylation of ERK1/2 without affecting the total abundance of the proteins (Fig. 6A). The activation of ERK1/2 was almost completely abolished by eplerenone and NAC contrasting with a less significant effect of apocynin (Fig. 6B).

Stimulation of snail-1 via ERK1/2. Snail, a zinc finger-containing transcription factor, has been shown to repress transcription of E-cadherin through binding to the E box elements in the E-cadherin promoter and to induce EMT (25). Real-time RT-PCR revealed a dose-dependent stimulatory effect of Aldo on snail-1 mRNA (Fig. 7A). The stimulation was significantly blocked by eplerenone, NAC, and rotenone but not apocynin, similar to the pattern of changes in ERK1/2, and the blockade was also achieved with U0126 (Fig. 7B).
Roles of mitochondrial ROS and ERK1/2 in Aldo-induced EMT. We determined the effects of rotenone and U0126 on Aldo-induced EMT in cultured HK-2 cells. As shown in Fig. 8, rotenone and U0126 both significantly inhibited Aldo-induced EMT as assessed by morphological change (A), real-time RT-PCR (B), and immunoblotting analysis (C) for α-SMA and E-cadherin expression.

EMT in DOCA salt-treated mice and effect of rotenone. A question arises as to whether the phenomenon observed in cell culture models indeed occurred in vivo. Therefore, we determined whether EMT took place in the kidney of DOCA salt-treated mice and whether rotenone exhibited a beneficial effect as predicted from the cell culture models. DOCA salt induced a 2.5-fold increase in α-SMA (Fig. 9A) and a 30% reduction of E-cadherin (Fig. 9B), as assessed by real-time RT-PCR. In line with this finding, immunofluorescence revealed that DOCA salt treatment induced a remarkable stimulation of α-SMA protein expression mostly in the renal epithelial cells (Fig. 9, C–E) and this stimulation was nearly completely inhibited by rotenone treatment (600 ppm in diet) and that E-cadherin exhibited exactly opposite gene expression patterns (Fig. 9, F–H). The rotenone-treated animals did not exhibit obvious toxicities as evaluated by histological analysis of several major organs including the brain, heart, liver, and kidney and by TUNEL assay (data not shown).

DISCUSSION

A large body of experimental evidence established the pathogenic role of Aldo in progression of renal injury. However, the site and mechanism of Aldo actions in the kidney are still incompletely understood. Nishiyama and co-workers (18) show that Aldo salt treatment induced glomerular injury associated with increased production of ROS in renal cortical tissues and this injury was significantly attenuated by tempol treatment. These findings establish an important role of ROS in Aldo salt-induced renal injury. A separate study from the same group of investigators provided further evidence for a direct stimulatory effect of Aldo on ROS production in cultured rat mesangial cells (15), suggesting that the glomerular cells may represent a potential target for Aldo-induced ROS. The present study extended this observation by identifying renal tubular epithelial cells as another important site for renal profibrotic actions of Aldo. We showed that exposure of human renal proximal tubular (HK-2) cells to Aldo for 48 h induced EMT as evidenced by conversion to the spindle-like morphology, loss of E-cadherin, and de novo expression of α-SMA. In line with this observation, we obtained evidence for EMT in the kidney of DOCA salt-treated mice. It is evident that EMT may represent an important mechanism for the renal deleterious effects of Aldo. This notion is compatible with the recently published evidence that EMT may play an important role in renal injury in several animal models.
recognized role of EMT in progression of various forms of renal injury. By utilizing a model of γGT-LacZ transgenic mice, which allows the indisputable identification of cells derived from proximal tubular epithelium in the kidney, Iwano et al. (9) recently demonstrated that more than one-third of renal interstitial myofibroblasts are derived from renal tubular epithelium via EMT.

MR has been suggested to mediate the pathogenic role of Aldo. We demonstrated the existence of MR in cultured HK-2 cells at both mRNA and protein levels. Furthermore, in response to Aldo treatment, MR was translocated from the cytosol to the nucleus in HK-2 cells, suggesting activation of MR. Blockade of MR with eplerenone remarkably inhibited Aldo-induced EMT. Taken together, the evidence obtained from the cell culture model suggests that MR is likely responsible for the profibrotic actions of Aldo in renal epithelial cells.

In line with the pivotal role of ROS in Aldo-induced renal injury, we observed a direct stimulatory effect of Aldo on ROS production in a human renal proximal tubular cell line. Subsequently, we attempted to determine the source of Aldo-induced ROS, e.g., the mitochondria electron-transfer chain vs. the NADPH oxidase using the mitochondrial respiratory chain complex I inhibitor rotenone and the NADPH oxidase inhibitor apocynin. Rotenone was highly effective not only in inhibiting Aldo-induced ROS production in cultured HK-2 cells but also in attenuating EMT in DOCA salt-treated mice. In contrast, apocynin was without an obvious effect on Aldo-induced ROS production. Also, Aldo treatment failed to elevate gene expression of gp91phox and p47phox in HK-2 cells. Taken together, these observations suggest that Aldo-induced ROS in HK-2 cells may be derived from mitochondria rather than NADPH oxidase. This notion is in contrast to a number of previous studies indicating NADPH oxidase as a source of Aldo-induced ROS production in rat mesangial cells (15) and rat ventricular cardiomyocytes (26). A possibility exists that Aldo utilizes different sources to generate ROS depending on individual cell types.

Given the important role of mitochondria in energy production, rotenone is suspected to induce some degree of toxicities. Therefore, we performed histological analysis of several internal organs as well as TUNEL assay on the kidney. These assays did not reveal obvious tissue injuries associated with rotenone treatment (data not shown). A study from National Toxicology Program reported that rotenone treatment for 14 days at 1,200 ppm or higher doses induced decreases in body weight gain in rats but not in mice and this dose range was even well tolerated for up to 2 yr. (1) It is unexpected that the relatively low dose and short duration of rotenone treatment in the present study will produce significant toxicities.
What would be the molecular targets of Aldo-induced ROS in renal epithelial cells? One of these targets appeared to be ERK1/2. We showed that Aldo treatment induced ERK1/2 activation that was significantly blocked by MR antagonist eplerenone, and by NAC and rotenone but not apocynin. We further demonstrated that the blockade of ERK1/2 with U0126 was able to prevent Aldo-induced EMT in HK-2 cells. It is likely that ERK1/2 acts downstream of mitochondrial-derived ROS in Aldo-induced signaling pathway in renal epithelial cells.

The Snail is a zinc finger transcription factor responsible for the loss of E-cadherin during EMT (3, 14, 34). We examined that possibility that the Snail may serve as a component in Aldo-induced signaling cascades in cultured renal epithelial cells. Indeed, Aldo treatment induced Snail-1 mRNA expression as early as 6 h preceding the occurrence of EMT. The stimulation was significantly blocked by eplerenone, NAC, and rotenone but not apocynin, similar to the pattern of changes in ERK1/2, suggesting that ERK1/2 and the Snail may both act downstream of mitochondrial-derived ROS. The effectiveness of U0126 in the inhibition of the Snail suggests that the transcription factor may serve as a downstream target of ERK1/2 in Aldo-induced cascades in HK-2 cells.

In summary, this study, for the first time, presents evidence that Aldo induces EMT in renal epithelial cells both in vitro and in vivo through MR-mediated, mitochondrial ROS-dependent activation of ERK1/2 and the Snail. These findings provide new insights into the renal deleterious effects of Aldo. The new information might be useful for the development of novel therapeutic strategies for renal fibrosis.

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**REFERENCES**