AMP-activated protein kinase inhibits TGF-β-, angiotensin II-, aldosterone-, high glucose-, and albumin-induced epithelial-mesenchymal transition

Jang Han Lee, Ji Hyun Kim, Ja Seon Kim, Jai Won Chang, Soon Bae Kim, Jung Sik Park, and Sang Koo Lee

Department of Internal Medicine, Asan Institute for Life Sciences, College of Medicine, University of Ulsan, Seoul, Korea

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AMP-activated protein kinase inhibits TGF-β-, angiotensin II-, aldosterone-, high glucose-, and albumin-induced epithelial-mesenchymal transition. Am J Physiol Renal Physiol 304: F686–F697, 2013. First published January 16, 2013; doi:10.1152/ajprenal.00148.2012.—The epithelial-mesenchymal transition (EMT) is a novel mechanism that promotes renal fibrosis. Transforming growth factor-β (TGF-β), angiotensin II, aldosterone, high glucose, and urinary albumin are well-known causes of EMT and renal fibrosis. We examined whether and how activation of AMP-activated protein kinase (AMPK) suppressed EMT induced by the above agents in tubular epithelial cells. All experiments were performed using HK-2 cells. Protein expression was measured by Western blot analysis. Intracellular reactive oxygen species (ROS) were analyzed by flow cytometry. Exposure of tubular cells to TGF-β (10 ng/ml), angiotensin II (1 μM), aldosterone (100 nM), high glucose (30 mM), and albumin (5 mg/ml) for 5 days induced EMT, as shown by upregulation of α-smooth muscle actin and downregulation of E-cadherin. ROS and NADPH oxidase 4 (Nox4) expression were increased, and antioxidants such as tiron and N-acetylcysteine inhibited EMT induction. Metformin (the best known clinical activator of AMPK) suppressed EMT induction through inhibition of ROS via induction of heme oxygenase-1 and endogenous antioxidant thioredoxin. An AMPK inhibitor (compound C) and another AMPK activator [5-aminoimidazole-4-carboxamide-1β (AICAR)] exerted the same effects as metformin. In conclusion, AMPK activation might be beneficial in attenuating the tubulointerstitial fibrosis induced by TGF-β, angiotensin II, aldosterone, high glucose, and urinary albumin.

AMPK; EMT; heme oxygenase-1; Nox4; ROS; thioredoxin

TUBULOINTERSTITIAL FIBROSIS is a final common pathway to end-stage chronic kidney diseases, and its severity correlates with renal prognosis. Emerging evidence suggests that tubular epithelial cells play a pivotal role in tubulointerstitial fibrosis through a process of epithelial-mesenchymal transition (EMT; Ref. 16).

EMT defines a phenotypic conversion of primary epithelial cells into mesenchymal cells, leading to morphological changes to fibroblastoid morphology; downregulation of epithelial marker proteins such as E-cadherin, zona occludens-1, and cytokeratin; and finally, upregulation of mesenchymal markers including α-smooth muscle actin (α-SMA), vimentin, and fibroblast-specific protein-1 (23).

It has been suggested that renal tubular epithelial cells can undergo EMT to become matrix-producing fibroblasts under pathologic conditions and participate in the pathogenesis of chronic renal diseases (16). Therefore, pharmacological prevention and/or reversal of EMT may serve as one of the possible therapeutic approaches to tubulointerstitial fibrosis.

EMT typically occurs in response to a number of environmental stresses and associated cytokine/growth factor stimuli. Transforming growth factor-β (TGF-β) has been described as the most potent inducer of fibrosis and EMT (13). Angiotensin II, aldosterone, high glucose, and urinary albumin, especially fatty acid-bearing albumin, are also well-known causes of EMT (2, 21, 34, 40).

It has been shown that EMT induced by the above agents is mediated through reactive oxygen species (ROS; Refs. 4, 19, 24, 30, 35).

Various ROS-producing systems exist in cells, and among them, the nicotinamide adenine dinucleotide phosphate [NAD(P)H] oxidase family has been identified as a major source of superoxide and hydrogen peroxide generation in kidney disease. Although all isoforms are potentially present in kidney cells, the constitutively active isofrom NADPH oxidase 4 (Nox4) is predominantly expressed, especially in mesangial and epithelial cells (11), and is now recognized as a key mediator of cell proliferation and matrix accumulation in renal disease (12). We examined the effect of EMT-inducing agents on the Nox4 expression in tubular epithelial cells.

It has been reported that AMP-activated protein kinase (AMPK) is involved in regulating many cellular functions including endothelial nitric oxide synthase activation, angiogenesis, and inhibition of vascular inflammation, although AMPK has traditionally been viewed as a modulator of metabolism (6). AMPK is also reported to inhibit cardiac and hepatic fibrosis (7, 36). However, whether and how AMPK suppresses TGF-β-, angiotensin II-, aldosterone-, high glucose-, and albumin-induced EMT in tubular epithelial cells, have not been investigated to date.

The best known clinical activator of AMPK is metformin, an antihyperglycemic agent used in type 2 diabetes (42). Therefore, we investigated the effects of metformin, followed by an AMPK inhibitor (compound C) and another AMPK activator [5-aminoimidazole-4-carboxamide-1β (AICAR)]. Heme oxygenase (HO) is a microsomal enzyme with inducible (HO-1) and constitutive (HO-2) isomers. HO catalyzes the breakdown of the heme moiety to generate cytoprotective products including bilirubin, ferritin, and carbon monoxide with effects against oxidative stress, inflammation, and apoptosis (1). Thioredoxin, a 12-kDa redox-sensitive disulfide oxidoreductase, is an important endogenous antioxidant and is ubiquitously expressed. It has been demonstrated that these two antioxidant systems can protect cells from ROS-induced oxidative damage (41, 38, 37). Therefore, we postulated that the inhibitory effect of AMPK on EMT induction might be mediated through inhibition of ROS via induction of HO-1 and thioredoxin.

METHODS

Reagent. TGF-β, angiotensin II, and glucose were obtained from R&D Systems (Minneapolis, MN). Aldosterone, fatty acids-bearing...
amp-activated protein kinase inhibits EMT

AMP-activated protein kinase (AMPK) is a crucial regulator of cellular energy homeostasis and plays a pivotal role in the prevention of epithelial-mesenchymal transition (EMT) in various pathophysiological conditions. EMT is a critical process involved in the development and maintenance of diverse tissues and organs, including the kidney, where it plays a role in the progression of renal fibrosis.

In the present study, we investigated the role of AMPK in the inhibition of EMT induced by transforming growth factor-β (TGF-β), angiotensin II (Ag II), aldosterone, glucose, and albumin. We used proximal tubular cells and observed that activation of AMPK by metformin or AICAR significantly inhibited EMT, as evidenced by the decreased expression of α-SMA and E-cadherin, two key markers of EMT.

1. **TGF-β**
   - Inhibition of TGF-β-induced EMT by AMPK activation
   - Cells were incubated with TGF-β (10 ng/ml) and treated with metformin or AICAR. Western blot analysis showed a significant decrease in α-SMA and E-cadherin expression in the AMPK-activated groups compared to the control.

2. **Ag II**
   - Ag II (10 ng/ml)
   - Similar to TGF-β, Ag II-induced EMT was also inhibited by AMPK activation.

3. **Aldosterone**
   - Aldosterone (100 nM)
   - Aldosterone-induced EMT was significantly reduced by AMPK activation.

4. **Glucose**
   - Glucose (30 mM)
   - Glucose-induced EMT was also inhibited by AMPK activation.

5. **Albumin**
   - Albumin (5 mg/ml)
   - Albumin-induced EMT was suppressed by AMPK activation.

**Cell culture and conditioning.** All experiments were performed using HK-2 cells, a human proximal tubular cell line. HK-2 cells were obtained from the American Type Culture Collection and have been characterized previously (29). The media were changed every 3 days until confluent. Cells were growth arrested in serum-free medium for 24 h before being used in experiments. Cells were incubated with TGF-β (10 ng/ml), protoporphyrin IX [Zn(II)PPIX] and the AMPK inhibitor (compound C) were obtained from Calbiochem (San Diego, CA).

**Fig. 1.** Inhibition of transforming growth factor-β (TGF-β; 1 ng/ml), angiotensin II (2 ng/ml), aldosterone (3 ng/ml), high glucose (4 mM), and albumin (5 mg/ml)-induced epithelial-mesenchymal transition (EMT) by activation of AMPK. Proximal tubular cells were incubated with TGF-β (10 ng/ml), angiotensin II (1 μM), aldosterone (100 nM), high glucose (30 mM), and albumin (5 mg/ml) for 3 days and then treated with metformin (1 mM), metformin with compound C (AMPK inhibitor, 20 μM), and 5-aminoimidazole-4-carboxamide-1β-riboside (AICAR; AMPK activator, 1 mM) for 2 days. Expression of α-smooth muscle actin (α-SMA) and E-cadherin was examined by Western blot analysis. Representative blots and quantitative analysis from 3 independent experiments are shown. Results are expressed as n-fold increase over control as means ± SE. Ag II, angiotensin II; aldo, aldosterone; glu, glucose; alb, albumin; met, metformin; comp C, compound C. ##P < 0.05 vs. TGF, Ag II, aldo, glu, alb. ###P < 0.05 vs. met.
angiotensin II (1 μM), aldosterone (100 nM), high glucose (30 mM), and albumin (5 mg/ml) for up to 5 days. For examining the effect of metformin, cells were incubated for 3 days and then treated with metformin (1 mM) for 2 days. The concentration of TGF-β, angiotensin II, aldosterone, high glucose, albumin, and metformin used in our experiment was based on the previous studies (39, 20, 2, 21, 27, 40).

**Assay of intracellular ROS.** Intracellular ROS production was detected by confocal scanning microscopy using 5-(and-6)-chloro- methyl-2',7'-dichlorodihydrofluorescein diacetate (CM-H2DCF-DA; Molecular Probes, Eugene, OR). CM-H2DCF-DA is a nonpolar compound that readily diffuses into cells, where it is hydrolyzed to the nonfluorescent polar derivative 2',7'-dichlorofluorescein (DCF) and thereby trapped within the cells. In the presence of a proper oxidant, DCFH is oxidized to the highly fluorescent 2',7'-dichlorofluorescein (DCF). Cells were incubated in the dark with 5 μM CM-H2DCF-DA for 30 min. After being washed three times, cells were examined with confocal scanning microscopy. (excitation, 488 nm; emission, 515–540 nm) To quantitate ROS generation, cells were resuspended and mean fluorescence intensity was measured using flow cytometry (FACScan; Becton Dickinson, San Jose, CA).

**Western blot analysis.** Equal amounts of protein from whole cell lysates were separated by a 10% SDS-polyacrylamide gels, transferred to nylon membrane. Membranes were incubated for 2 h with primary antibody, followed by peroxidase-conjugated secondary an-
Antibody-antigen complexes were detected with ECL plus chemiluminescence (Amersham Pharmacia Biotech, Arlington, IL). The band intensities were quantified using a GS-710 densitometer and QuantityOne software (Bio-Rad, Hercules, CA).

**Immunofluorescence.** Cells grown on coverslips were fixed with 4% paraformaldehyde for 15 min, permeabilized with 0.2% Triton X-100, and then blocked with 2% BSA in PBS for 1 h. Cells were incubated with primary antibody against α-SMA or E-cadherin for overnight. Then, the specimens were washed with PBS and incubated with FITC-conjugated secondary antibody for 1 h at room temperature. Nuclei were stained with 4',6-diamidino-2-phenylindole. After being washed with PBS, coverslips were mounted in 80% glycerol in PBS and photographed using confocal microscope.

**Small interfering RNA transfection.** Transfection of small interfering (si)RNA was performed with Lipofectamine 2000, according to the manufacturer’s instructions (Invitrogen, Carlsbad, CA). Cells were transfected with siRNA against α-AMPK (Ambion) at 100 pmol/ml for 6 h in serum-free medium, and then culture medium was changed to normal medium containing 10% FBS for 24 h. Nonspecific siRNA was used as negative control. The efficiency of AMPK siRNA was evaluated by Western blotting of the p-AMPK and p-ACC. Transfected cells were pretreated with metformin or AICAR and then

![Diagram of immunofluorescence results](http://ajprenal.physiology.org/)
incubated with albumin (5 mg/ml) for 3 days. Western blotting for α-SMA was performed.

Statistical analysis. Data are expressed as means ± SE. A Kruskall-Wallis test was used for comparison of more than two groups, followed by a Mann-Whitney U-test for comparison using a microcomputer-assisted program with SPSS for Window 10.0 (SPSS, Chicago, IL). A P value <0.05 was considered significant.

RESULTS

TGF-β, angiotensin II, aldosterone, high glucose, and albumin induced EMT in tubular epithelial cells. To determine whether TGF-β, angiotensin II, aldosterone, high glucose, and albumin induced EMT, we examined the change of two EMT biomarkers, upregulation of α-SMA and downregulation of E-cadherin.

![Fig. 4. Time-dependent induction of NADPH oxidase 4 (Nox4) by TGF-β, angiotensin II, aldosterone, high glucose, and albumin. Proximal tubular cells were incubated with TGF-β (10 ng/ml; 1), angiotensin II (1 μM; 2), aldosterone (100 nM; 3), high glucose (30 mM; 4), and albumin (5 mg/ml; 5) for indicated times (0–5 days). Western blotting for Nox4 was performed. Representative blots (A) and quantitative analysis at day 5 (B) from 3 independent experiments are shown. #P < 0.05 vs. con.](http://ajprenal.physiology.org/)

![Fig. 5. Effect of metformin, compound C, and AICAR on the phosphorylated (p)-AMPK and p-acetyl-CoA carboxylase (ACC). Proximal tubular cells were incubated with metformin (1 mM), metformin with compound C (AMPK inhibitor, 20 μM), AICAR (AMPK activator, 1 mM), and AICAR with compound C for 6 h. Expression of p-AMPK (A) and its key downstream signaling molecule p-ACC (acetyl-CoA carboxylase; B) were examined by Western blot analysis. Representative blots and quantitative analysis from 3 independent experiments are shown. Results are expressed as n-fold increase over control as means ± SE. #P < 0.05 vs. con. ###P < 0.05 vs. met or AICAR.](http://ajprenal.physiology.org/)
Exposure of tubular cells to TGF-β (10 ng/ml), angiotensin II (1 μM), aldosterone (100 nM), high glucose (30 mM), and albumin (5 mg/ml) for 5 days induced EMT as shown by upregulation of α-SMA and downregulation of E-cadherin (Fig. 1).

ROS were involved in TGF-β-, angiotensin II-, aldosterone-, high glucose-, and albumin-induced EMT. Exposure of tubular cells to TGF-β (10 ng/ml), angiotensin II (1 μM), aldosterone (100 nM), high glucose (30 mM), and albumin (5 mg/ml) for 5 days induced ROS generation (Fig. 2).

To determine whether EMT induction was mediated through ROS, we examined the effect of antioxidants (10 mM tiron and 20 mM N-acetylcysteine). Western blot revealed that both tiron and N-acetylcysteine inhibited the EMT induction (Fig. 3).

Nox4 expression was induced by TGF-β, angiotensin II, aldosterone, high glucose, and albumin. To determine whether TGF-β, angiotensin II, aldosterone, high glucose, and albumin induced Nox4 expression, we performed Western blot analysis.

Fig. 6. Immunofluorescence study showing that albumin-induced upregulation of α-SMA and downregulation of E-cadherin were inhibited by activation of AMPK. Proximal tubular cells were incubated with albumin (5 mg/ml) for 3 days and then treated with metformin (1 mM), metformin with compound C (AMPK inhibitor, 20 μM), and AICAR (AMPK activator, 1 mM) for 2 days. Immunofluorescence staining for α-SMA and E-cadherin were performed. Representative microscopic scans are shown.

Fig. 7. Effects of transfection with nonspecific (control) or AMPK small interfering (si)RNA. A: inhibition of metformin-induced p-AMPK and p-ACC by AMPK siRNA. Cells were transfected with nonspecific (control) or AMPK siRNA. Then, cells were treated with metformin (1 mM) for 6 h. Expression of p-AMPK and p-ACC was examined by Western blot analysis. B: suppression of metformin’s and AICAR’s inhibitory effect on the albumin-induced α-SMA by AMPK siRNA. Cells were transfected with nonspecific (control) or AMPK siRNA. Then, cells were pretreated with metformin or AICAR, followed by albumin (5 mg/ml) for 3 days. Expression of α-SMA was examined by Western blot analysis. Representative blots and quantitative analysis from 3 independent experiments are shown. Results are expressed as n-fold increase over control as means ± SE. AIC, AICAR, #P < 0.05 vs. con. ##P < 0.05 vs. alb (albumin). ###P < 0.05 vs. met or AICAR.
Western blot analysis revealed that Nox4 expression was increased in a time-dependent manner for up to 5 days (Fig. 4).

Activation of AMPK inhibited TGF-β, angiotensin II, aldosterone, high glucose, and albumin-induced EMT. To confirm whether metformin and AICAR could be used as an AMPK activator and compound C as an AMPK inhibitor in our HK-2 cells, we examined the effects of metformin (1 mM), AICAR (1 mM), and compound C (20 μM) on the p-AMPK and its key downstream signaling molecule p-ACC. Western blot revealed that metformin and AICAR induced p-AMPK and p-ACC. Compound C inhibited the metformin- and AICAR-induced p-AMPK and p-ACC (Fig. 5). Metformin (1 mM) suppressed TGF-β, angiotensin II, aldosterone, high glucose, and albumin-induced EMT, as shown by inhibition of upregulation of α-SMA and downregulation of E-cadherin.

The AMPK inhibitor (20 μM compound C) blocked the effect of metformin and another AMPK activator (1 mM AICAR) exerted the same effects as metformin (Fig. 1).

In agreement with the Western blot data, immunofluorescence staining also revealed that metformin and AICAR inhibited the albumin-induced upregulation of α-SMA and downregulation of E-cadherin. Compound C blocked the effect of metformin (Fig. 6).

To confirm the role of AMPK in metformin-induced inhibition of EMT, we knocked down AMPK expression by siRNA transfection. As expected, AMPK siRNA inhibited the metformin-induced p-AMPK and p-ACC but not nonspecific siRNA (Fig. 7A). The inhibitory effects of metformin and AICAR on the albumin-induced α-SMA were blocked by transfection with an AMPK siRNA. Nonspecific siRNA had no

Fig. 8. Inhibition of TGF-β (1), angiotensin II (2), aldosterone (3), high glucose (4), and albumin (5)-induced Nox4 expression by activation of AMPK. Proximal tubular cells were incubated with TGF-β (10 ng/ml), angiotensin II (1 μM), aldosterone (100 nM), high glucose (30 mM), and albumin (5 mg/ml) for 3 days and then treated with metformin (1 mM), metformin with compound C (AMPK inhibitor, 20 μM), and AICAR (AMPK activator, 1 mM) for 2 days. Western blotting for Nox4 was performed. Representative blots and quantitative analysis from 3 independent experiments are shown. Results are expressed as n-fold increase over control as means ± SE. #P < 0.05 vs. con. ##P < 0.05 vs. TGF, Ag II, aldo, glu, alb. ###P < 0.05 vs. met.
significant effect (Fig. 7B). These data suggested that effects of metformin and AICAR were mediated by a process involving AMPK.

Effect of AMPK on the EMT was mediated through suppression of ROS and Nox 4 expression. To determine how AMPK activation inhibited EMT induction, we examined the effect of metformin, compound C, and AICAR on the ROS generation and Nox4 expression. Metformin (1 mM) reduced TGF-β-, angiotensin II-, aldosterone-, high glucose-, and albumin-induced intracellular ROS generation and Nox4 expression. Compound C blocked the effect of metformin and AICAR exerted the same effects as metformin (Fig. 2 and Fig. 8).

Inhibitory effect of AMPK on the EMT was mediated via induction of HO-1 and thioredoxin. Metformin (1 mM) induced the expression of HO-1 and thioredoxin. Compound C (20 μM) blocked the metformin-induced HO-1, and thioredoxin expression. AICAR (1 mM) increased the HO-1 and thioredoxin expression like metformin (Fig. 9).

![Fig. 9](image-url) Induction of heme oxygenase-1 (HO-1) and thioredoxin by activation of AMPK. Proximal tubular cells were incubated with TGF-β (10 ng/ml), angiotensin II (1 μM), aldosterone (100 nM), glucose (30 mM), and albumin (5 mg/ml) for 3 days and then treated with metformin (1 mM), metformin with compound C (AMPK inhibitor, 20 μM), and AICAR (AMPK activator, 1 mM) for 6 h. Western blotting for HO-1 and thioredoxin was performed. Representative blots and quantitative analysis from 3 independent experiments are shown. Results are expressed as n-fold increase over control as means ± SE. *P < 0.05 vs. con, **P < 0.05 vs. TGF, Ag II, aldo, glu, alb. ***P < 0.05 vs. met.
To determine whether upregulation of HO-1 and thioredoxin by metformin was directly involved in metformin’s inhibitory effect on the EMT, we examined the effect of an HO-1 inhibitor [20 mM Zn(II)PPIX] and thioredoxin inhibitor (25 μM PX12). Both Zn(II)PPIX and PX12 reversed the metformin’s inhibitory effect on the EMT (Fig. 10).

As expected, both Zn(II)PPIX and PX12 blocked the inhibitory effect of metformin on ROS generation as well (Fig. 11).

DISCUSSION

The present study demonstrates that activation of AMPK inhibits TGF-β-, angiotensin II-, aldosterone-, high glucose- and albumin-induced EMT through suppression of ROS via induction of HO-1 and thioredoxin. These results suggest that AMPK activation may be beneficial in attenuating the tubulointerstitial fibrosis induced by the above agents.

EMT is a process by which differentiated epithelial cells undergo a phenotypic conversion that gives rise to the matrix-producing fibroblasts and myofibroblasts. It has been demonstrated that up to one-third of all disease-related fibroblasts can originate from tubular epithelia at the site of injury through EMT (16), suggesting that EMT is a one of the major mechanisms of tubulointerstitial fibrosis.

Of the many factors that trigger EMT, TGF-β is the most important and well studied (13). Angiotensin II, aldosterone, high glucose, and urinary protein are also well-known causes of renal fibrosis and induce EMT in tubular epithelial cells (2, 21, 34, 40).

TGF-β, angiotensin II, aldosterone, and high glucose are known to induce ROS generation and Nox4 expression (4, 24, 30, 35), although the effect of albumin on the Nox4 expression in tubular epithelial cells has not been studied as yet. We found that albumin also increased the Nox4 expression in tubular epithelial cells.

Accumulating evidence suggests that targeting EMT may serve as one of the possible therapeutic approaches to tubulointerstitial fibrosis.

We found that metformin (the best known clinical activator of AMPK) suppressed the TGF-β-, angiotensin II-, aldosterone-, high glucose- and albumin-induced EMT through inhibition of ROS via induction of HO-1 and endogenous antioxidant thioredoxin. AMPK inhibitor (compound C) blocked the effect of metformin and another AMPK activator (AICAR) exerted the same effects as metformin.

In addition, to confirm the role of AMPK in metformin-induced inhibition of EMT, we performed the siRNA experi-

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**Fig. 10.** Reversal of metformin’s inhibitory effect on TGF-β (1)-, angiotensin II (2)-, aldosterone (3)-, high glucose (4)-, and albumin (5)-induced EMT by HO-1 inhibitor (PPIX) and thioredoxin inhibitor (PX12). Proximal tubular cells were incubated with TGF-β (10 ng/ml), angiotensin II (1 μM), aldosterone (100 nM), high glucose (30 mM), and albumin (5 mg/ml) for 3 days and then treated with metformin (1 mM), metformin with HO-1 inhibitor (PPIX, 20 μM) and metformin with thioredoxin inhibitor (PX 12, 25 μM) for 2 days. Western blotting for α-SMA and E-cadherin was performed. Representative blots from 3 independent experiments were shown.
Another major antioxidant system in the body is thioredoxin and chronic renal insults (8, 14, 18). The thioredoxin system is ubiquitously expressed, is stress inducible, and defends against cellular oxidative stress (37). It has been reported that metformin induces expression of HO-1 and thioredoxin in endothelial cells (15, 22). Similarly, we found that activation of AMPK induced expression of both HO-1 and thioredoxin in tubular epithelial cells. Furthermore, we found that the inhibitory effect of AMPK on the EMT was mediated via induction of HO-1 and thioredoxin by showing that the inhibitor of HO-1 and thioredoxin blocked the effect of metformin on the EMT.

AMPK is abundantly expressed in the kidney where it is involved in a variety of pathophysiological processes. Regarding kidney diseases, it has been suggested that AMPK activation provides therapeutic benefits in acute renal ischemia (26), diabetic nephropathy (20), renal cystogenesis (31), and experimental gentamycin-induced nephropathy (25). Therefore, our results, together with other reports, suggest that AMPK activation may have therapeutic potential in a variety of renal diseases.

In conclusion, our study provides data suggesting that AMPK activation may serve as a promising therapeutic target in the prevention and/or treatment of tubulointerstitial fibrosis induced by TGF-β, angiotensin II, aldosterone, high glucose, and urinary albumin.

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

Fig. 11. Suppression of metformin’s inhibitory effect on TGF-β, angiotensin II, aldosterone, high glucose- and albumin-induced ROS generation by HO-1 inhibitor (PPIX) and thioredoxin inhibitor (PX12). Proximal tubular cells were incubated with TGF-β (10 ng/ml; 1), angiotensin II (1 μM; 2), aldosterone (100 nM; 3), high glucose (30 mM; 4), and albumin (5 mg/ml; 5) for 3 days and then treated with metformin (1 mM), metformin with HO-1 inhibitor (PPIX, 20 μM), and metformin with thioredoxin inhibitor (PX12, 25 μM) for 2 days. DCF-sensitive cellular ROS were measured using flow cytometry. Quantitative analysis from 3 independent experiments are shown. Results are expressed as mean channel fluorescence ± SE. *P < 0.05 vs. con. **P < 0.05 vs. TGF, Ag II, aldo, glu, alb. ***P < 0.05 vs. met.

ment against AMPK because several studies reported that compound C also inhibited other kinase signaling pathways independently of AMPK inhibition such as inhibition of the hypoxic activation of hypoxia-inducible factor-1 (10, 33). In our study, transfection with an AMPK siRNA blocked the inhibitory effects of metformin and AICAR on the albumin-induced α-SMA, suggesting that effects of metformin and AICAR were mediated by a process involving AMPK.

In support of our findings, Cufí et al. (5) had reported that activation of AMPK inhibited TGF-β-induced EMT in breast cancer stem cells. Furthermore, we found that the inhibitory effect of AMPK on the EMT was mediated through suppression of ROS generation. These findings were consistent with the previous reports that metformin significantly reduced intracellular ROS levels (17, 28) and Nox4 expression (9, 17).

Mammalian cells have developed several protective mechanisms to prevent ROS formation. These mechanisms employ low-molecular-mass antioxidants such as ascorbic acid, glutathione and tocopherols; ROS-interacting enzymes such as superoxide dismutase, peroxidases, and catalases; and redox regulation enzymes (32). Among the various protective enzymes, extensive studies suggest that HO-1 plays an important role in maintaining antioxidant homeostasis during oxidative stress (3). HO-1 is the key enzyme for heme metabolism, which exhibits potent antioxidant, antiapoptotic activity and thus plays an important role in the protection of cells against various stresses, such as ROS (1, 38, 41). Furthermore, it has been suggested that HO-1 induction is protective in many acute and chronic renal insults (8, 14, 18). The thioredoxin system is another major antioxidant system in the body. Thioredoxin is
AMP-Activated Protein Kinase Inhibits EMT

AUTHOR CONTRIBUTIONS


REFERENCES


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