Activation of AMP-activated protein kinase inhibits ER stress and renal fibrosis

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Am J Physiol Renal Physiol 308: F226–F236, 2015. First published November 26, 2014; doi:10.1152/ajprenal.00495.2014.—It has been suggested that endoplasmic reticulum (ER) stress facilitates fibrotic remodeling. Therefore, modulation of ER stress may serve as one of the possible therapeutic approaches to renal fibrosis. We examined whether and how activation of AMP-activated protein kinase (AMPK) suppressed ER stress induced by chemical ER stress inducers [tunicamycin (TM) and thapsigargin (TG)] and also nonchemical inducers in tubular HK-2 cells. We further investigated the in vivo effects of AMPK on ER stress and renal fibrosis. Western blot analysis, immunofluorescence, small interfering (si)RNA experiments, and immunohistochemical staining were performed. Metformin (the best known clinical activator of AMPK) suppressed TM- or TG-induced ER stress, as shown by the inhibition of TM- or TG-induced upregulation of glucose-related protein (GRP)78 and phosphorylated eukaryotic initiation factor-2α through induction of heme oxygenase-1. Metformin inhibited TM- or TG-induced epithelial-mesenchymal transitions as well. Compound C (AMPK inhibitor) blocked the effect of metformin, and 5-aminoimidazole-4-carboxamide-1β riboside (another AMPK activator) exerted the same effects as metformin. Transfection with siRNA targeting AMPK blocked the effect of metformin. Consistent with the results of cell culture experiments, metformin reduced renal cortical GRP78 expression and increased heme oxygenase-1 expression in a mouse model of ER stress-induced acute kidney injury by TM. Activation of AMPK also suppressed ER stress by transforming growth factor-β, ANG II, aldosterone, and high glucose. Furthermore, metformin reduced GRP78 expression and renal fibrosis in a mouse model of unilateral ureteral obstruction. In conclusion, AMPK may serve as a promising therapeutic target through reducing ER stress and renal fibrosis.

AMP-activated protein kinase; endoplasmic reticulum stress; fibrosis; heme oxygenase-1; metformin

The endoplasmic reticulum (ER) is the cellular organelle responsible for the biosynthesis, folding, assembly, and modification of proteins. ER stress refers to physiological or pathological states that result in the accumulation of misfolded proteins in the ER caused by environmental changes such as ROS, aberrant regulation of Ca2+, glucose deprivation, etc. (6). ER stress triggers an unfolded protein response (UPR), which adapts to the changing environment and reestablishes normal ER function through enhancing translational attenuation, increasing the production of redox proteins and molecular chaperones, and upregulating the production of protein degradation enzymes through the activation of three major sensors known as PKR-like ER kinase, activating transcription factor-6, and inositol-requiring enzyme (IRE)-1. Glucose-related protein (GRP)78 is a central regulator of ER homeostasis, and phosphorylation of eukaryotic initiation factor (eIF)2α leads to attenuate the translation initiation rate, both of which are biomarkers for ER stress (37).

However, excessive ER stress triggers apoptosis through a signal transduction cascade including C/EBP homologous protein (CHOP) and apoptosis signal-regulating kinase-1 (49). Furthermore, ER stress and UPR can also contribute to other ER stress-independent cellular responses (29).

Although ER stress serves a protective role that allows cells to survive from the noxious stimuli, prolonged ER stress contributes to the development and progression of many chronic diseases, including neurodegenerative disorders, diabetes, atherosclerosis, and cancer (33). Therefore, the development of therapies to reduce ER stress may yield effective treatment strategies.

With respect to fibrosis, it has been suggested that ER stress and UPR pathways facilitate fibrotic remodeling through the activation of proapoptotic pathways, induction of the epithelial-mesenchymal transition (EMT), and promotion of inflammatory responses (43). Prolonged ER stress may lead to fibrosis through the activation of CHOP-mediated apoptosis followed by an inflammatory response and the release of profibrotic cytokines (26). ER stress is able to induce inflammatory responses through the activation of NF-κB and MAPK JNK (50), induction of cytokines such as interferon-β and IL-23 (12, 39), and activation of the NLRP3 inflammasome (30), which regulates the maturation and secretion of proinflammatory cytokines such as IL-1β and IL-18 (41). ER stress seems to play an important role in albuminuria-induced inflammasome activation (10). Furthermore, UPR is known to promote the release of transforming growth factor (TGF)-β and expression of collagen I (17).

Therefore, it has been reported that inhibition of ER stress by 4-phenylbutyric acid, an ER stress inhibitor, protects against carbon tetrachloride-induced hepatic fibrogenesis (48), isoproteenol-induced cardiac fibrosis (2), and unilateral ureteral obstruction (UUO)-induced renal fibrosis (4). Similarly, CHOP deficiency is also known to attenuate cholesterol-induced liver fibrosis (44).

Thus, prevention and/or reversal of ER stress may serve as one of the possible therapeutic approaches to renal fibrosis.

AMP-activated protein kinase (AMPK) is an evolutionarily conserved serine/threonine kinase that is ubiquitously expressed in mammalian tissues, including the kidney, and acts as a major regulator of cellular and whole body energy homeostasis (40). Therefore, AMPK has traditionally been viewed as a modulator of metabolism. However, it has been reported that activation of AMPK is also able to modulate ER stress, suggesting that AMPK could be a novel therapeutic target for the treatment of ER stress-induced injury.

We previously reported that activation of AMPK by metformin could inhibit albumin-induced ER stress in tubular epithelial cells and a protein-overload proteinuria rat model.
(23). In the present study, we extended our experiments to determine whether and how activation of AMPK suppressed ER stress induced by chemical ER stress inducers such as tunicamycin (TM) and thapsigargin (TG) as well as nonchemical inducers such as TGF-β, ANG II, aldosterone, and high glucose in tubular epithelial cells.

The best-known clinical activator of AMPK is metformin, an antihyperglycemic agent widely used for the treatment of type 2 diabetes. Therefore, we investigated the effects of metformin followed by an AMPK activator [5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR)] on ER stress in tubular cells. To confirm the role of AMPK in the suppression of ER stress, we investigated the effects of small interfering (si)RNA targeting AMPK as well. We further investigated the involvement of AMPK in the suppression of ER stress, we investigated the effects of AMPK activator [5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR)] on ER stress in tubular cells. To confirm the role of AMPK in the suppression of ER stress, we investigated the effects of small interfering (si)RNA targeting AMPK as well. We further investigated the involvement of AMPK in the suppression of ER stress.

**METHODS**

**Reagents.** TGF-β, ANG II, and glucose were obtained from R&D Systems (Minneapolis, MN), TM, TG, aldosterone, metformin, and AICAR were obtained from Sigma Chemical (St. Louis, MO). Antibodies to α-smooth muscle actin (α-SMA), E-cadherin, GRP78, and monocyte chemotactic protein-1 were acquired from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies to CHOP, heme oxygenase (HO)-1, total eIF2α, phosphophosphorylated eIF2α (Ser51), and hortensid phosphorylase-conjugated secondary antibody were purchased from Cell Signaling Technology (Beverly, MA). Zinc protoporphyrin IX (Zn(II)PPIX) and AMPK inhibitor (compound C) were obtained from Calbiochem (San Diego, CA).

**Cell culture and conditioning.** All experiments were performed using HK-2 cells, a human proximal tubular cell line (35). HK-2 cells were obtained from the American Type Culture Collection. Media were changed every 3 days until confluent. Cells were grown until confluent. Cells were cultured in serum-free medium for 24 h before being used in experiments.

To determine whether activation of AMPK suppressed TM- or TG-induced ER stress, cells were incubated with TM (0.2 μM) or TG (0.2 μM) with or without metformin, compound C, and AICAR for 24 h.

In our previous report (24), we demonstrated that metformin and AICAR could induce the activation of phosphorylated (p-)AMPK and its key downstream signaling molecule p-acetyl CoA carboxylase (p-ACC) in our HK-2 cells. Compound C inhibited metformin- and AICAR-induced p-AMPK and p-ACC.

To determine whether activation of AMPK suppressed TGF-β-, ANG II-, aldosterone-, and high glucose-induced ER stress, cells were incubated with TGF-β (10 ng/ml), ANG II (1 μM), aldosterone (100 nM), and high glucose (30 mM) for 3 days and then treated with or without metformin, compound C, and AICAR for 2 days.

**Fig. 1.** Inhibition of tunicamycin (TM)- or thapsigargin (TG)-induced endoplasmic reticulum (ER) stress by activation of AMP-activated protein kinase (AMPK). Proximal tubular cells were incubated with TM (0.2 μM) or TG (0.2 μM) with or without metformin (Met; 1 mM), compound C (Comp; AMPK inhibitor, 20 μM), and 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR or AIC; another AMPK activator, 1 mM) for 24 h. Expression of glucose-related protein (GRP78) and phosphorylated (p-)eukaryotic initiation factor (eIF)2α was examined by Western blot analysis. Representative blots and quantitative analysis from three independent experiments are shown. Results are expressed as mean ± SE of fold increases over control (Con). ##P < 0.05 vs. Con; ###P < 0.05 vs. TM or TG; ####P < 0.05 vs. TM + Met, TM + AIC, TG + Met, or TG + AIC.
The concentrations of TM, TG, TGF-β, ANG II, aldosterone, high glucose, albumin, and metformin used in our experiments were based on previous studies (24, 32).

Western blot analysis. Equal amounts of protein from whole cell lysates were separated by 10% SDS polyacrylamide gels and transferred to nylon membranes. Membranes were incubated for 2 h with primary antibody followed by peroxidase-conjugated secondary antibody. Antibody-antigen complexes were detected with ECL plus chemiluminescence (Amersham Pharmacia Biotech, Arlington, IL). 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, E-cadherin, and GRP78 for overnight. Specimens were then washed with PBS, coverslips were mounted in 80% glycerol in PBS and photographed using confocal microscope.

siRNA transfection. Transfection of siRNA was performed with Lipofectamine 2000 according to the manufacturer’s instructions (Invitrogen, Carlsbad, CA). Cells were transfected with siRNA against α1-AMPK (Ambion) at 100 pmol/ml for 6 h in serum-free medium, and the culture medium was then changed to normal medium containing 10% FBS for 24 h.

Nonspecific siRNA was used as a negative control. Transfected cells were pretreated with metformin or AICAR and then incubated with TM or TG for 24 h. Western blot analysis for GRP78 and α-SMA was performed. The efficiency of AMPK siRNA on metformin-induced p-AMPK and p-ACC in HK-2 cells was demonstrated in our previous report (24).

Experimental mouse model of ER stress-induced acute kidney injury by TM. Male mice (C57BL/6) weighing ~20 g were given free access to water and standard chow. ER stress-induced acute kidney injury in the mouse was induced by a single intraperitoneal injection of TM (2 mg/kg). This model has been previously characterized (14). Mice were randomly divided into three groups: control mice (n = 4), mice with TM injection (n = 4), and mice with TM injection plus metformin (n = 4). Metformin was administered by gavage at 300 mg·kg⁻¹·day⁻¹ (25). Mice were euthanized after 3 days. Serum creatinine was measured at the time of death. Semiquantitative evaluation of tubular damage including necrosis and degeneration on a scale from 0 to 4 was performed (14).

Experimental mouse model of UUO-induced progressive kidney injury. For the induction of UUO, male mice (C57BL/6) weighing ~20 g were anesthetized, and the left ureter was ligated through a flank incision. Mice were randomly divided into three groups: control mice (n = 4), mice with UUO (n = 4), and mice with UUO plus metformin (n = 4). Metformin was administered by gavage at 300 mg·kg⁻¹·day⁻¹. On the 14th day after UUO surgery, mice were euthanized. The kidney was fixed in 4% buffered formalin and embedded in paraffin for histological evaluation. Tubulointerstitial fibrosis was graded by degree of interstitial collagen deposition using Masson trichrome-stained sections (Trichrome Stain Kit, Sigma), where 0% no staining, 1% 25% staining, 2% 25–50% staining, 3% 50% to <75% staining, and 4% 75–100% staining of the sections (4). All animal protocols were approved by the Institutional Animal Care and Use Committee at our institution.

Fig. 2. Immunofluorescence experiments showing suppression of TM-induced upregulation of GRP78 and α-smooth muscle actin (α-SMA) and downregulation of E-cadherin by activation of AMPK. Proximal tubular cells were incubated with TM (0.2 μM) with or without Met (1 mM), Comp (AMPK inhibitor, 20 μM), and AIC (another AMPK activator, 1 mM) for 24 h. Immunofluorescence staining for GRP78, α-SMA, and E-cadherin was performed. Representative microscopic scans are shown.

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approved by the Institutional Animal Care and Use Committee of Asan Institute for Life Sciences.

**Immunohistochemical staining.** Kidney sections were deparaffinized in xylene and rehydrated in graded ethanol. Endogenous peroxidase activity was quenched in absolute methanol plus 0.3% (vol/vol) hydrogen peroxide for 30 min. Antigen was retrieved by heating the sections in 10 mM citrate buffer (pH 6.0) in a microwave oven. Nonspecific binding was blocked in 1% BSA. Sections were then incubated with primary antibody for GRP78 and HO-1 for 2 h followed by biotinylated secondary antibodies for 1 h and horseradish peroxidase-streptavidin conjugate for 30 min. Labeling was visualized with chromogen diaminobenzidine stain (Dako, Glostrup, Denmark). Finally, slides were counterstained with hematoxylin.

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

**RESULTS**

**Activation of AMPK suppressed ER stress induced by chemical ER stress inducers such as TM and TG in tubular epithelial cells.** To determine whether activation of AMPK suppressed TM- or TG-induced ER stress in tubular HK-2 cells, we examined the change of two ER stress biomarkers: upregulation of GRP78 and p-eIF2α.

Metformin (1 mM) suppressed TM- or TG-induced ER stress, as shown by the inhibition of TM- or TG-induced upregulation of GRP78 and p-eIF2α. Compound C (AMPK inhibitor, 20 μM) blocked the effect of metformin, and AICAR (another AMPK activator, 1 mM) exerted the same effects as metformin (Fig. 1).

Consistent with the data of Western blot analysis, immunofluorescence staining revealed that metformin inhibited the TM-induced upregulation of GRP78, which was abolished by pretreatment with compound C. AICAR also suppressed the TM-induced upregulation of GRP78 (Fig. 2). These results suggest that inhibition of ER stress by metformin is mediated through the activation of AMPK.

**Activation of AMPK also inhibited TM- or TG-induced EMTs.** To determine whether activation of AMPK inhibited TM- or TG-induced EMTs, we examined the change of two EMT biomarkers: upregulation of α-SMA and downregulation of E-cadherin.

Metformin (1 mM) suppressed TM- or TG-induced EMTs, as evidenced by the inhibition of TM- or TG-induced upregulation of α-SMA and downregulation of E-cadherin. Compound C (AMPK inhibitor, 20 μM) blocked the effect of

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**Fig. 3.** Suppression of TM- or TM-induced epithelial-mesenchymal transitions (EMTs) by activation of AMPK. Proximal tubular cells were incubated with TM (0.2 μM) or TG (0.2 μM) with or without Met (1 mM), Comp (AMPK inhibitor, 20 μM), and AIC (another AMPK activator, 1 mM) for 24 h. Expression of α-SMA and E-cadherin was examined by Western blot analysis. Representative blots and quantitative analysis from three independent experiments are shown. Results are expressed as mean ± SE of fold increases over Con. **##P < 0.05 vs. TM or TG; ###P < 0.05 vs. TM + Met, TM + AIC, TG + Met, or TG + AIC.**
Metformin and AICAR (another AMPK activator, 1 mM) exerted the same effects as metformin (Fig. 3).

In agreement with the data of Western blot analysis, immunofluorescence staining revealed that metformin inhibited the TM-induced upregulation of α-SMA and downregulation of E-cadherin, which were reversed by pretreatment with compound C. AICAR exerted the same effects as metformin (Fig. 2).

To confirm the role of AMPK in suppressing ER stress and EMT, we examined the effects of siRNA targeting AMPK. Transfection with siRNA targeting AMPK blocked the inhibitory effects of metformin on the TM- or TG-induced upregulation of GRP78 and α-SMA. Nonspecific siRNA had no significant effect (Fig. 4).

The inhibitory effect of AMPK on TM- or TG-induced ER stress was mediated through induction of HO-1. Metformin (1 mM) increased the expression of HO-1, which was prevented by compound C. AICAR also increased the expression of HO-1 (Fig. 5), suggesting that activation of AMPK could induce the expression of HO-1.

To determine whether upregulation of HO-1 by metformin was directly involved in metformin’s inhibitory effect on TM- or TG-induced ER stress, we examined the effect of the HO-1 inhibitor Zn(II)PPiX (20 μM). Western blot analysis revealed that Zn(II)PPiX reversed metformin’s inhibitory effect on TM- or TG-induced ER stress. The inhibitory effect of AICAR was also blocked by Zn(II)PPiX (Fig. 6), suggesting that the inhibitory effect of AMPK on ER stress was mediated through HO-1.

**Metformin reduced the renal tubular expression of GRP78 and increased HO-1 expression in a mouse model of ER stress-induced acute kidney injury by TM.** To demonstrate in vivo inhibitory effect of metformin on TM-induced ER stress, we performed animal experiments using a mouse model of ER stress-induced acute kidney injury by TM. In this model, it has been reported that male mice show a high induction of ER stress markers such as GRP78 and CHOP with proximal tubular damages in the outer cortex (14).

Consistent with the results of cell culture experiments, metformin reduced the renal tubular expression of GRP78 and...
increased HO-1 expression. Western blot analysis of renal cortical tissue showed the same results (Fig. 7, A and B). Metformin also reduced serum creatinine levels and tubular damage (Fig. 7C).

Activation of AMPK suppressed ER stress induced by nonchemical ER stress inducers such as TGF-β, ANG II, aldosterone, and high glucose as well. To determine whether activation of AMPK suppressed TGF-β, ANG II, aldosterone-, and high glucose-induced ER stress, we examined the change of three ER stress biomarkers: upregulation of GRP78, p-eIF2α, and CHOP.

Metformin (1 mM) suppressed TGF-β, ANG II, aldosterone-, and high glucose-induced ER stress, as shown by the inhibition of upregulation of GRP78, p-eIF2α, and CHOP expression. Compound C (AMPK inhibitor, 20 μM) blocked the effect of metformin, and AICAR (another AMPK activator, 1 mM) exerted the same effects as metformin (Fig. 8).

Metformin reduced the renal tubular expression of GRP78 and also renal fibrosis in a mouse model of UUO. The UUO model is the well-established model of renal progressive injury. It has been suggested that TGF-β, ANG II and aldosterone, play an important role in the initiation of UUO-induced renal fibrosis (34, 47). Furthermore, it has been reported that activation of ER stress is associated with UUO-induced renal apoptosis and fibrosis (4).

Therefore, we used a mouse model of UUO to test the hypothesis that activation of AMPK by metformin could reduce ER stress and renal fibrosis.

Metformin reduced the renal tubular expression of GRP78 and increased HO-1 expression in the UUO mouse model (Fig. 9A). Western blot analysis of renal cortical tissue showed the same results (Fig. 9B). Furthermore, metformin reduced renal fibrosis as well, as shown by Masson trichrome stain (Fig. 10A). Metformin also reduced the expression of collagen type I and monocyte chemotactic protein-1 (Fig. 10B).

DISCUSSION

The present study demonstrated that activation of AMPK suppressed TM- or TG-induced ER stress through the induction of HO-1 in tubular epithelial cells. AMPK also inhibited TM- or TG-induced EMTs. Consistent with the results of cell culture experiments, metformin reduced renal cortical GRP78 expression and increased HO-1 expression in a mouse model of ER stress-induced acute kidney injury by TM. Activation of AMPK also suppressed ER stress induced by TGF-β, ANG II,
aldosterone, and high glucose. Furthermore, metformin reduced GRP78 expression and renal fibrosis in the UUO mouse model. These results suggest that activation of AMPK may serve as a future pharmacological target in kidney diseases by reducing ER stress and renal fibrosis.

Tubulointerstitial fibrosis has been recognized as a key process in the progression of renal disease. However, the specific mechanism linking tubular cells to tubulointerstitial fibrosis has not been completely revealed.

Recently, ER stress and UPR pathways are emerging as important factors in the development of organ fibrosis through the activation of proapoptotic pathways, induction of EMT, and promotion of inflammatory responses (43). As ER stress is increasingly identified as a mediator of fibrotic remodeling, blockade of ER stress may offer a promising therapeutic approach in treating ER stress-mediated fibrosis.

AMPK is a crucial regulator of energy metabolic homeostasis. Besides its metabolic effects, it has been suggested that activation of AMPK is able to inhibit ER stress induced by various agents in different cells (45, 9, 19, 46).

It has been reported that AMPK protects cardiomyocytes against hypoxic injury through attenuation of ER stress (45) and suppresses oxidized LDL- or palmitate-induced ER stress in endothelial cells and liver cells (9, 19). In the kidney, we and Thériault et al. (23, 46) have previously reported that metformin attenuated albumin- and glucosamine-induced ER stress, respectively, in renal epithelial cells.

In the present study, we found that activation of AMPK could suppress ER stress induced by chemical ER stress inducers such as TM and TG as well as nonchemical inducers such as TGF-β, ANG II, aldosterone, and high glucose in tubular epithelial cells. Animal studies have also shown that

Fig. 7. Reduction of renal tubular GRP78 expression and induction of HO-1 expression by Met in a mouse model of ER stress-induced acute kidney injury by TM. The ER stress-induced acute kidney injury model was induced by a single intraperitoneal injection of TM (2 mg/kg). Mice were randomly divided into three groups: Con mice (n = 4), mice with TM injection (n = 4), and mice with TM injection plus Met for 3 days (n = 4). Met was administered by gavage at 300 mg·kg⁻¹·day⁻¹. Immunohistochemical staining (A) and Western blot analysis of renal cortical tissue (B) for GRP78 and HO-1 were performed. Serum creatinine levels and semiquantitative scores of tubular damage were measured (C). Representative microscopic scans and quantitative analysis are shown. #P < 0.05 vs. Con; ##P < 0.05 vs. TM.
metformin reduced renal tubular ER stress protein (GRP78) expression in both ER stress-induced acute kidney injury mouse and UUO mouse models. Similar to the results of our study, it has been reported that the inhibitory effects of atorvastatin on homocysteine-induced ER stress are mediated through activation of AMPK (15).

However, the mechanisms of how activation of AMPK inhibits ER stress are not well defined. One of the possible mechanisms is that inhibitory effects of AMPK on ER stress may be mediated through inhibition of NAD(P)H oxidase-derived ROS (9). ROS not only directly oxidize and damage DNA, proteins, and lipids but also activate several stress signaling pathways and cause cellular injury and dysfunction. Therefore, mammalian cells have developed several protective mechanisms to prevent ROS formation. Among them, HO-1 plays an important role in maintaining antioxidant homeostasis during oxidative stress (7). HO-1 is the key enzyme involved in the degradation of heme to iron, carbon monoxide, and biliverdin, which have antioxidant, antiapoptotic, and anti-inflammatory properties, resulting in cytoprotection against various stresses, such as ROS (38).

Metformin is known to reduce both intracellular ROS levels and NADPH oxidase 4 expression (16). Furthermore, it has been reported that AMPK can stimulate the expression of HO-1 in macrophages and endothelial cells via the induction of nuclear factor-erythroid 2-related factor 2 (28). We have also previously reported that AMPK is able to suppress ROS via the nuclear factor-erythroid 2-related factor 2 (28). We have also previously reported that AMPK is able to suppress ROS via the nuclear factor-erythroid 2-related factor 2 (28). Therefore, we examined whether the inhibitory effect of AMPK on ER stress was mediated through HO-1.

We found that activation of AMPK induced the expression of HO-1 and that an inhibitor of HO-1 blocked the inhibitory effect of AMPK on ER stress in tubular epithelial cells, suggesting that the inhibitory effect of AMPK on ER stress was mediated through HO-1. Animal studies have also shown that metformin could increase the expression of HO-1. In support of our findings, it has been reported that the HO-1 system may be potentially therapeutic in vascular diseases associated with ER stress (22). Furthermore, it has been suggested that HO-1 induction is protective in many acute and chronic renal insults (8, 11, 21).
Another suggested mechanism by which HO-1 suppresses ER stress is that carbon monoxide, a reaction product of HO-1, may be able to attenuate ER stress-induced activation of IRE-1, activating transcription factor-6, and CREBH (5). Activation of the IRE-1 arm of UPR is also known to be involved in the ER stress-induced EMT in tubular epithelial cell (32), suggesting a possible link among ER stress, IRE-1, and the EMT.

Although HO-1 was presented as a downstream target of AMPK in our study, it was interesting to note that HO-1 per se was able to induce AMPK, possibly by increasing adiponectin production (3). Whether a positive feedback regulation between AMPK and HO-1 exists in tubular cells warrants further investigation.

In the present study, we showed that activation of AMPK could suppress ER stress induced by both chemical and nonchemical inducers. However, it is not yet clear whether activation of AMPK could have therapeutic benefits by reducing renal fibrosis. We demonstrated that activation of AMPK by metformin was able to reduce ER stress and renal fibrosis in a mouse model of UUO. Similar to our study, Satriano et al. (36) reported that induction of AMPK activity with either metformin or AICAR corrected metabolic inefficiency, improved renal function, and ameliorated kidney fibrosis and structural alterations in the subtotal nephrectomy model of chronic kidney disease.

In addition, it has been reported that suppression of liver fibrosis by berberine as well as inhibition of myocardial fibrosis by a 3-hydroxy-3-methylglutaryl CoA reductase inhibitor is mediated through activation of AMPK (13, 27).

Regarding kidney diseases, it has been suggested that activation of AMPK is able to provide therapeutic benefits in acute renal ischemia (31), diabetic nephropathy (1, 25), renal podocyte injury in type 2 diabetes (20), polycystic kidney disease (42), and high-fat diet-induced renal injury (18).

Together with these reports, our data suggest that activation of AMPK may have clinical therapeutic potential in a variety of kidney diseases.

In conclusion, our study provides clues suggesting that activation of AMPK can be a novel therapeutic target in kidney disease by reducing ER stress and renal fibrosis.

**GRANTS**

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Fig. 9. Reduction of renal GRP78 expression and induction of HO-1 expression by Met in a mouse model of unilateral ureteral obstruction (UUO). The UUO mouse model was induced by ligation of the left ureter through a flank incision. Mice were randomly divided into three groups: Con mice (n = 4), mice with UUO (n = 4), and mice with UUO plus Met (n = 4) for 14 days. Met was administered by gavage at 300 mg·kg⁻¹·day⁻¹. Immunohistochemical staining (A) and Western blot analysis of renal cortical tissue (B) for GRP78 and HO-1 were performed. Representative microscopic scans and quantitative analysis are shown. #P < 0.05 vs. Con; ##P < 0.05 vs. UUO.
A Masson trichrome stain for fibrosis

control mouse  mouse with UUO  mouse with UUO + metformin

B Western blot of renal cortical tissue for collagen I and MCP-1

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

AUTHOR CONTRIBUTIONS

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12. Goodall JC, Wu C, Zhang Y, McNeill L, Ellis L, Saudek V, Gaston JS. Endoplasmic reticulum stress-induced transcription factor, CHOP, is cru-

Fig. 10. Reduction of renal fibrosis by Met in a mouse model of UUO. The UUO mouse model was induced by ligation of the left ureter through a flank incision. Mice were randomly divided into three groups: Con mice (n = 4), mice with UUO (n = 4), and mice with UUO plus Met (n = 4) for 14 days. Met was administered by gavage at 300 mg·kg⁻¹·day⁻¹. Scores of interstitial collagen deposition using Masson trichrome-stained sections were measured (A). Western blot analysis of renal cortical tissue for collagen type I and monocyte chemotactic protein (MCP)-1 was performed (B). Representative microscopic scans and quantitative analysis are shown. #P < 0.05 vs. Con; ##P < 0.05 vs. UUO.
AMPK INHIBITS ER STRESS AND RENAL FIBROSIS

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MINIREVIEW

The signaling pathways activated by energy stress-induced protein kinase A (AMPK) provide unique opportunities for the development of targeted therapies for endoplasmic reticulum (ER)-stress-related renal disease.

The role of endoplasmic reticulum stress and death decisions.


