Uric acid-induced phenotypic transition of renal tubular cells as a novel mechanism of chronic kidney disease

Eun-Sun Ryu,1 Mi Jin Kim,1 Hyun-Soo Shin,1 Yang-Hee Jang,1 Hack Sun Choi,1 Inho Jo,2 Richard J. Johnson,3 and Duk-Hee Kang1

1Division of Nephrology, Department of Internal Medicine, Ewha Womens University School of Medicine, Ewha Medical Research Center, Seoul, Korea; 2Department of Molecular Medicine, Ewha Womens University School of Medicine, Ewha Medical Research Center, Seoul, Korea; and 3Division of Renal Diseases and Hypertension, University of Colorado-Denver, Aurora, Colorado

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Ryu ES, Kim MJ, Shin HS, Jang YH, Choi HS, Jo I, Johnson RJ, Kang DH. Uric acid-induced phenotypic transition of renal tubular cells as a novel mechanism of chronic kidney disease. Am J Physiol Renal Physiol 304: F471–F480, 2013. First published January 2, 2013; doi:10.1152/ajprenal.00560.2012.—Recent experimental and clinical studies suggest a causal role of uric acid in the development of chronic kidney disease. Most studies have focused on uric acid-induced endothelial dysfunction, oxidative stress, and inflammation in the kidney. The direct effects of uric acid on renal tubular cells have not been studied in detail, and whether uric acid can mediate phenotypic transition of renal tubular cells such as epithelial-to-mesenchymal transition (EMT) is not known. We therefore investigated whether uric acid could alter E-cadherin expression and EMT in the kidney of hyperuricemic rats and in cultured renal tubular cells (NRK cells). Experimental hyperuricemia was associated with evidence of EMT before the development of significant tubulointerstitial fibrosis at 4 wk, as shown by decreased E-cadherin expression and an increased α-smooth muscle actin (α-SMA). Allopurinol significantly inhibited uric acid-induced changes in E-cadherin and α-SMA with an amelioration of renal fibrosis at 6 wk. In cultured NRK cells, uric acid induced EMT, which was blocked by the organic anion transport inhibitor probenecid. Uric acid increased expression of transcriptional factors associated with decreased synthesis of E-cadherin (Snail and Slug). Uric acid also increased the degradation of E-cadherin via ubiquitination, which is of importance since downregulation of E-cadherin is considered to be a triggering mechanism for EMT. In conclusion, uric acid induces EMT of renal tubular cells decreasing E-cadherin synthesis via an activation of Snail and Slug as well as increasing the degradation of E-cadherin.

uric acid; chronic kidney disease; renal tubular cells; epithelial-to-mesenchymal transition; E-cadherin

HYPERURICEMIA IN CHRONIC KIDNEY disease (CKD) has long been viewed as a consequence of decreased renal function rather than a risk factor for the development or progression of renal disease (8). However, recent epidemiologic studies suggest that uric acid per se might predict the development of kidney disease in healthy individuals and subjects with impaired renal function (2, 9, 18, 35, 42, 50). Pilot treatment studies also reported that lowering serum uric acid levels decreased blood pressure and retarded the aggravation of renal function in patients with established kidney disease (13, 25, 46). However, further clinical trials are necessary before uric acid-lowering therapies are implemented in subjects with CKD. In addition, more basic science evidence is also needed to identify the potential mechanism(s) by which uric acid may induce renal disease.

Many factors involved in the causation of renal disease have direct effects on renal tubules. Some profibrogenic factors induce phenotypic transition of renal tubules in vitro, which is termed epithelial-to-mesenchymal transition (EMT). EMT is characterized by a decrease in the expressions of E-cadherin and zona occludens protein (ZO-1) with an acquisition of de novo α-smooth muscle actin (α-SMA) expression and is associated with the development of renal fibrosis (29, 30, 55). EMT has also been demonstrated in vivo by Strutz et al. (47) as well as others (14, 22, 52, 56), although this has been challenged by some investigators (14, 55). Various stimuli such as high glucose, hypoxia, profibrotic cytokines, and inflammatory mediators are known to trigger the signals to activate transcription factors for downregulation of the expression of epithelial cell markers in tubular epithelial cells, which in turn induce phenotypic transformation (12, 17). Importantly, EMT is one of the earliest phenomena of renal fibrosis and has been considered a therapeutic target due to its reversible characteristic (12, 14, 30).

To further understand the mechanism of uric acid-associated kidney disease, we investigated the effect of uric acid on phenotypic transition of renal tubules in an animal model of hyperuricemia and also cultured renal tubular cells with an elucidation of the mechanism of E-cadherin downregulation. We also examined whether uric acid-lowering therapy ameliorated uric acid-induced EMT in the kidney.

MATERIALS AND METHODS

Reagents. All chemicals and tissue culture plates were obtained from Sigma-Aldrich (St. Louis, MO) and Nunc Labware (Waltham, MA), unless otherwise stated. Hyperuricemia was induced by feeding the rats with the diet containing 2% oxonic acid (OA; hepatic uricase inhibitor; Sigma). Allopurinol (xanthine oxidase inhibitor; Schein Pharmaceutical, Florham Park, NJ) was dissolved in drinking water at a concentration of 13 mg/dl. Uric acid (Ultrapure, 1–6 mg/dl; Sigma) was dissolved in warmed media and filtered. Crystals were not detectable (polarizing microscopy) nor did they develop during cell incubation. Endotoxin was not detectable in all batches of uric acid (Limulus Amebocyte Assay; BioWhittaker, Walkersville, MD), which indicated <0.015 endotoxin U/ml. Mycoplasma contamination was also excluded (Immu-Mark Myco-Test; ICN Biomedicals, Irvine, CA). The uric acid level of control media containing 5% FBS was below the detection limit.
(0.05 vs. 0 and 2 wk. # 0.05 vs. 0, 2, and 4 wk.)

**Renal morphology and immunohistochemistry.** Tissue for light microscopy and immunoperoxidase staining was fixed in methyl Carnoy’s solution and embedded in paraffin. Four-micrometer sections were stained with the periodic acid-Schiff (PAS) reagent and counterstained with hematoxylin. Indirect immunoperoxidase staining of 4-μm sections was performed as described previously (26), with monoclonal antibodies directed to the following antigens: E-cadherin with mouse monoclonal (BD Biosciences, Franklin Lakes, NJ), α-smooth muscle actin (α-SMA; Sigma), vimentin (Millipore, Billerica, MA), and collagen III (Sigma). Controls included omitting the primary antibody and substitution of the primary antibody with preimmune mouse serum. All analyses were performed blinded. The tubulointerstitial fibrosis score (0–5) was evaluated on the basis of PAS staining as described previously (26). Percent positive area of the expression of E-cadherin, α-SMA, and vimentin in the tubules of renal cortex was analyzed using computer image analyzer (OPTIMAS 6.5; MediaCybernetics, Bothell, WA). Vascular α-SMA and glomerular vimentin expressions were excluded in analyzing the positive area for α-SMA and vimentin.

**Cell morphology and fluorescent immunocytochemistry.** Rat renal tubular epithelial cells (NRK-52E cells) were obtained from the American Type Culture Collection (ATCC, Manassas, VA). NRK-52E cells were cultured in DMEM supplemented with 5% FBS, 60 μg/ml penicillin. Cells were maintained at 37° in a humidified 5% CO2-95% air atmosphere incubator. Fresh growth medium was added to the cells every 2 days until cells reached an adequate confluency for each experiment. The uric acid concentration for the in vitro experiments was determined by the consideration of normal level of uric acid in rats and the results of the cell proliferation and lactate dehydrogenase (LDH) assay. Uric acid at the concentrations of 1–6 mg/dl did not induce the changes in cell proliferation and LDH release (data not shown). After the stimulation of cells with uric acid for 1–4 days, morphological changes of the NRK-52E cells were examined under an inverted phase contrast microscope (Axiovert 200; Carl Zeiss, Oberkochen, Germany), and the images were obtained by digital camera (AxioCam HRC; Carl Zeiss). For immunoﬂuorescence staining, cells were washed and fixed in 4% phosphate-buffered...
paraformaldehyde (25 min at room temperature) and permeabilized with 0.2% Triton X-100 in PBS (15 min at room temperature). After being washed with PBS and blocking with 5% BSA for 1 h, cells were incubated with primary antibodies specific for E-cadherin as a marker of epithelial cell or α-SMA as mesenchymal cell marker in 5% BSA overnight at 4°C, followed by an incubation with goat anti-mouse IgG-FITC-conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h at room temperature in the dark. The nucleus was counterstained with DAPI, and the cells were visualized under the Axiovert 200 fluorescence microscope (Carl Ziss).

Western blot analysis. Protein samples isolated from cell lysate or homogenates of renal cortex were mixed in reducing buffer, boiled, resolved on 10% SDS-PAGE gels, and transferred to a polyvinylidene difluoride membrane by electroblotting. Membranes were blocked in 5% wt/vol nonfat milk powder in TBS for 30 min at room temperature. Then, blots were incubated overnight in blocking solution with primary antibodies at 4°C. Antibodies to E-cadherin (BD Biosciences, Franklin Lakes, NJ), α-SMA, Snail (Cell Signaling Technology, Beverly, MA), Slug (Santa Cruz Biotechnology), and Twist (Santa Cruz Biotechnology) were used. After the blot was washed with TBS with Tween 20, the blots were incubated with horseradish peroxidase-conjugated secondary antibodies corresponding to each primary antibody followed by enhanced chemiluminescence detection (Santa Cruz Biotechnology). Positive immunoreactive bands were quantified by densitometry and compared with the expression of human β-actin.

Real-time PCR. The levels of transcripts were determined by real-time PCR (RT-PCR) on the ABI PRISM 7000 sequence detection system using SYBR Green I as a double-stranded DNA-specific dye according to the manufacturer’s instructions (Applied Biosystems, Foster City, CA, USA). The PCR reaction was carried out in 5 μM of CDNA, 10 μM of SYBR Green PCR master mix, and 5 pM of sense and antisense primers of E-cadherin (forward primer: AACGAGGGCATTCTGAAAACA and reverse primer: CACTGTC ACGTGCAGAATGTACTG) or α-SMA (forward primer: GACCCTGAAGTATCCGATAGAACA and reverse primer: CACGCGAAGCTCGTTA TAGAAG) for a final volume of 20 μM per reaction. Primer concentrations were determined by preliminary experiments that analyzed the optimal concentrations of each primer. The relative mRNA expression levels of the target genes in each sample were calculated using the comparative CT method. The CT value is the cycle number at which the fluorescence signal is greater than a defined threshold. At least three independent PCR procedures were performed to allow statistical analysis. The amount of PCR products was normalized with the housekeeping gene β-actin to determine the relative expression ratios for each mRNA in relation to the control group.

Detection of E-cadherin ubiquitination and degradation. NRK-52E cells were seeded overnight in 5% FBS/DMEM, and then cells were rinsed twice with PBS before switching to uric acid-containing DMEM. At 24 and 48 h of uric acid exposure, whole cell lysates were subjected to immunoprecipitation using an anti-E-cadherin antibody. Ubiquitinated E-cadherin was detected by Western blot with anti-ubiquitin antibody.

Statistical analysis. All data are presented as means ± SD. Differences in parameters at each time point and concentration of uric acid were compared by paired t-test. Differences in the various parameters between groups were evaluated by two-way ANOVA followed by correction for multiple comparisons. Significance was defined as P < 0.05.

RESULTS

Serum uric acid, BUN, creatinine, and renal pathology in OA-treated rats. The administration of the uricase inhibitor OA (2%) to rats resulted in a mild increase in serum uric acid level from 2.00 to 2.22 mg/dl at 4 wk (2.48 vs. 0.97 mg/dl, OA vs. control; P = 0.05; Fig. 1A). Blood urea nitrogen and creatinine levels were significantly increased at 6 wk in the hyperuricemic rats and were ameliorated with allopurinol treatment (Fig. 1, B and C). The kidneys of OA-treated rats

![Fig. 2. Renal pathology in hyperuricemic rats. In OA-fed rats, there were no significant morphologic changes except mild glomerular hypertrophy at 4 wk (B) compared with control rats (A). Linear interstitial fibrosis with tubular dilatation and shrinkage became evident from 6 wk (C), which was markedly ameliorated in allopurinol (Allo)-treated rats (D). Collagen type III immunohistochemistry revealed mild interstitial fibrosis at 6 wk (G) compared with control (E), OA 4 wk (F), OA 6 wk + allopurinol (H). Magnification: ×100 (A–D) and ×200 (E–H).](http://ajprenal.physiology.org/)

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showed no significant pathologic changes on PAS staining at 4 wk; however, focal interstitial fibrosis with tubular dilatation and shrinkage was observed at 6 wk of OA treatment (Fig. 2). Interstitial fibrosis was evident in immunohistochemistry of type III collagen (Fig. 2, E–G). An increase in tubulointerstitial fibrosis score in hyperuricemic rats was prevented in rats treated with allopurinol (Fig. 1D and 2).

**EMT in the kidneys of OA-treated rats: expression of E-cadherin, α-SMA, and vimentin.** The expression of the epithelial cell marker E-cadherin was evident both in cell membrane and cytoplasm in renal cortical tubules of normal rats as shown in Fig. 3A. OA-treated rats demonstrated a decrease in E-cadherin expression at 4 wk when tubulointerstitial changes were not evident yet (Fig. 3B). A decrease in E-cadherin expression was more evident in proximal tubules, which was identified by the presence of brush border and a lower number of nuclei per unit area (denoted P in Fig. 3A); however, E-cadherin expression was also decreased in other cortical tubules in hyperuricemic rats (percent positive area of E-cadherin in renal cortex; 48.5 ± 11.9 vs. 12.5 ± 5.5%, control vs. hyperuricemic rats; \( P < 0.01 \)). At the same time, some tubules showed de novo staining of α-SMA and vimentin (Fig. 3, F and J), the markers of mesenchymal cells. These findings suggested uric acid-induced phenotypic transition of renal tubular cells before the development of significant tubulointerstitial fibrosis. At 6 wk of OA treatment, an altered expression of epithelial and mesenchymal cell markers became more evident (Fig. 3, C, G, and K), which were inhibited by allopurinol treatment (Fig. 3, D, H, and L). Interestingly, the expression of α-SMA and vimentin was found both in renal tubules and in the interstitium, suggesting an accumulation or migration of myofibroblasts into interstitial areas. Consistent with immunohistochemistry staining, Western blot of the renal cortex showed an altered expression of E-cadherin and α-SMA (Fig. 4), suggesting the presence of EMT before the development of significant tubulointerstitial fibrosis. Allopurinol significantly ameliorated uric acid-induced changes in E-cadherin and α-SMA.

**Effect of uric acid on cell morphology of NRK cells.** Uric acid (6 mg/dl) induced the changes in cell morphology of NRK cells from 24 h of stimulation from a typical cobblestone shape to elongated spindle-shaped cells with a loss of cell-to-cell contact (Fig. 5), which became more evident with prolonged exposure to uric acid.

**Effect of uric acid on the expression of epithelial and mesenchymal cell markers.** In association with morphologic transition, we also examined the expression of E-cadherin as a marker of epithelial cell and α-SMA as an indicator of mesenchymal phenotype. There was abundant cellular expression of E-cadherin primarily at the cell membrane in unstimulated renal tubular cells (Fig. 6A) whereas both membranous and cytoplasmic E-cadherin expressions were significantly decreased in uric acid-stimulated cells (Fig. 6B). In addition, uric acid induced de novo expression of α-SMA (Fig. 6D) in contrast to almost negative staining in untreated cells (Fig. 6C). Western blot analysis also demonstrated uric acid-induced alteration in the expression of E-cadherin and α-SMA.

![Fig. 3. Phenotypic transition of renal tubules in hyperuricemic rats. Representative immunohistochemistry of E-cadherin (A–D), α-smooth muscle actin (α-SMA; E–H), and vimentin (J–L) staining in control rats (A, E, and I) and rats in 4 wk (B, F, and J) and 6 wk (C, G, and K) of OA feeding. E-cadherin staining in renal tubules was markedly decreased in proximal tubules (P) and other cortical tubules shown as either faint cytoplasmic or membranous staining in 4 wk (B) and 6 wk (C) compared with control rats (A). α-SMA was expressed in vascular smooth muscle of arteriole (arrowheads) in control (E) and hyperuricemic rat kidney (F and G). However, some tubular cells expressed α-SMA in cytoplasm of renal tubules (F, arrows) and interstitial area (G, arrows). Vimentin was also expressed in tubulointerstitial area in hyperuricemic rats (J and K) in contrast to control rats. Allopurinol treatment ameliorated an altered expression of E-cadherin (D), α-SMA (H), and vimentin (L) in hyperuricemic rats (6 wk).](http://ajprenal.physiology.org/)

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Fig. 4. Expression of E-cadherin and α-SMA in renal cortex. Representative Western blot (A) and its quantitation analysis (B) demonstrated a decreased E-cadherin expression in 4 wk of OA feeding (OA4) with an increased α-SMA, which was more evident in 6 wk of OA (OA6). Allopurinol ameliorated the changes in E-cadherin and α-SMA. *P < 0.05 vs. control (C) and OA6. #P < 0.05 vs. others. †P < 0.05 vs. C and OA6 + Allo.

Fig. 5. Effect of uric acid on cell morphology in NRK-52E cells. NRK-52E cells displayed typical cobblestone appearance at different stages of confluency from day 1 to day 6 after seeding (A–D). Uric acid (6 mg/dl) induced a dissociation of cell adhesion at day 1 (E), day 2 (F), and day 3 (G) followed by a phenotypic transition to elongated fibroblast-like cells from cuboidal clustered epithelial cells, which was more evident at day 4 (H). Magnification: ×100.
With 3 mg/dl of uric acid, the expressions of E-cadherin were decreased to ~40% of those in control cells at 48 h (P < 0.05 vs. control) (Fig. 7, A and D). In addition, uric acid markedly upregulated α-SMA expression from 48 h in a dose- and time-dependent manner (P < 0.05 vs. control; Fig. 7, A, B, D, and E). Importantly, pretreatment with probenecid (1 mM) significantly blocked the uric acid-induced changes in the expression of E-cadherin and α-SMA in NRK-52E cells (P < 0.05 vs. uric acid-treated cells; Fig. 7, C and F), suggesting that uric acid per se entering into cells was responsible for uric acid-induced EMT. Consistent with the changes in protein expression of E-cadherin in uric acid-stimulated renal tubular cells, mRNA expression of E-cadherin was decreased in NRK cells 6 h (Fig. 8A). There was also a significant increase in α-SMA mRNA expression in NRK cells exposed to uric acid at 6 and 24 h (Fig. 8, C and D).

Effect of uric acid on the expression of Snail, Slug, Twist, and E-cadherin degradation. To understand the mechanism of uric acid-induced downregulation of E-cadherin, we examined the changes in the expression of Snail, Slug, and Twist, which are major transcription factors of E-cadherin. Uric acid increased the expression of Snail and Slug whereas it did not induce the change in Twist expression (Fig. 9A). Uric acid also increased the degradation of E-cadherin (Fig. 9B). E-cadherin degradation products, shown as two lower molecular weight protein bands, were found in uric acid-stimulated cells. We next tested whether E-cadherin was ubiquitinated on uric acid stimulation. Significant ubiquitination of immunoprecipitated E-cadherin was observed in NRK cells exposed to uric acid for 24 h, suggesting uric acid-induced E-cadherin degradation via ubiquitination can be another mechanism of E-cadherin downregulation in addition to a decreased transcription of E-cadherin.

**DISCUSSION**

The novel finding of our study is that uric acid induces phenotypic changes in cultured tubular epithelial cells and in the renal tubules of an animal model of hyperuricemia. Previous studies from our group and others demonstrated uric acid-induced endothelial dysfunction and local inflammation in the kidney as major mechanisms of renal disease (6, 11, 26, 31, 54). This study suggests that uric acid also has direct effects on renal tubules as another potential mechanism of uric acid-induced renal disease. Probenecid, which blocks the entry of soluble uric acid into cells, inhibited uric acid-induced EMT, and allopurinol treatment also ameliorated an alteration of E-cadherin and α-SMA expression in OA-treated animal, which suggested uric acid per se was responsible for phenotypic transition of renal tubular cells.

Experimental and clinical studies support a role for uric acid in the progression of renal disease independent of urate crystal deposition or hypertension (19, 26). Epidemiological studies have shown that serum uric acid is an independent risk factor for the development of CKD. In one Japanese study, hyperuricemia conferred a 10.8-fold increased risk in women and a 3.8-fold increased risk in men for the development of CKD compared with those with normal uric acid levels (21). The higher relative risk in the hyperuricemic subject was indepen-
dent of age, body mass index, blood pressure, total cholesterol, serum albumin, glucose, and proteinuria. Previous studies have suggested that hyperuricemia may induce renal injury by caus-

ing afferent arteriolar thickening and rarely hyalinosis of the

preglomerular arterioles of the kidney, often accompanied by
glomerular hypertrophy (26, 31, 33). Further studies showed
that uric acid induced endothelial dysfunction via an activation
of local renin-angiotensin system with an induction of oxida-

![Graph](image)

**Fig. 7.** Dose- and time-dependent effect of uric acid on the expression of E-cadherin and α-SMA in NRK-52E cells. Representative western blot demonstrated uric acid-induced alteration in E-cadherin and α-SMA (A and B). Uric acid induced a dose-dependent changes in the expressions of E-cadherin and α-SMA from 4 to 6 h (n = 5). Probenecid (P) significantly inhibited uric acid-induced changes in E-cadherin and α-SMA (C). Quantitative analysis is shown in D–F. Con, control; UA, uric acid. *P < 0.05 vs. 0 and 1 mg/dL of uric acid; #P < 0.05 vs. day 0 and day 1; †P < 0.05 vs. others.

![Graph](image)

**Fig. 8.** Effect of uric acid on mRNA expression of E-cadherin and α-SMA in NRK-52E cells. E-cadherin mRNA expression was significantly decreased at 6 h (A) and 24 h (B) in uric acid-stimulated cells whereas α-SMA mRNA was upregulated at 6 h (C) and 24 h (D, n = 5). *P < 0.05 vs. 0 and 1 mg/dL of uric acid. #P < 0.05 vs. 0, 1, and 3 mg/dL of uric acid.
tive stress and local inflammation (27, 28, 49, 54). Collectively, uric acid-induced renal disease has been explained by endothelial dysfunction, which is associated with glomerular hypertension with a reduction in renal plasma flow that could lead to renal injury (43).

To date, few studies have considered whether there could be direct tubular effects of uric acid. One study showed uric acid-induced inhibition of tubular cell proliferation in vitro (16) and a second study demonstrated an upregulation of ERK, BAX, and α-SMA in HK-2 cells exposed to uric acid using SILAC coupled to liquid chromatography-mass spectrometry (39). Therefore, our observation of the early development of EMT of renal tubules provides an important insight regarding the mechanism of progression of renal disease in hyperuricemia. In this study, phenotypic transition of renal tubules was found before the development of renal dysfunction and significant tubulointerstitial fibrosis in hyperuricemic rats and was ameliorated by uric acid-lowering therapy. After 4 wk of OA treatment, the expression of α-SMA and vimentin was observed in the cytoplasm of some renal tubules along with a decrease in E-cadherin expression, suggesting a phenotypic transition to mesenchymal cells from epithelial cells, and it became evident at the interstitial area with the development of fibrosis in 6 wk. In cultured renal tubular cells, a loss of cell adhesion was the first finding observed as early as 24 h of uric acid exposure, which became evident with a morphologic change into elongated fibroblastoid cells. Phenotypic transition of NRK cells was associated with de novo acquisition of α-SMA as well as a significant decrease in E-cadherin.

Although our data suggest uric acid is responsible for the EMT of renal tubules, the possibility of OA-related phenotype transition in animal model of hyperuricemia cannot be ruled out. However, our in vitro data showed OA (up to 1 mM of concentration) did not induce EMT in several different renal tubular cell lines (data now shown). In addition, the beneficial effect of the xanthine oxidase inhibitor allopurinol could be attributed to lowering uric acid or other effects of allopurinol including its antioxidant action. Furthermore, we have previously reported that both xanthine oxidase inhibitors and uricosuric agents can ameliorate renal fibrosis in the hyperuricemic remnant kidney model (26). Therefore, our data suggest that it is not OA but rather uric acid that is responsible for EMT, and lowering uric acid by inhibiting uric acid synthesis or by enhancing uric acid excretion alleviates EMT of renal tubules.

E-cadherin downregulation was originally regarded as a marker of EMT; however, it is now thought to be an important initial step in the transdifferentiation of epithelial cells to a mesenchymal phenotype (3, 37). Expression of E-cadherin is a hallmark of a fully differentiated epithelium where it functions to maintain cell-cell junctions, thereby inhibiting aberrant cell proliferation and migration. Indeed, a loss of E-cadherin function via small interfering RNA-mediated knockdown resulted in loss of cell contacts and cell scattering with an acquisition of elongated, fibroblast-like morphology in mammary epithelial cells (15, 34, 37, 51). Importantly, genetic inactivation of E-cadherin per se induced an expression of mesenchymal protein such as N-cadherin and vimentin (37). Genetic or epigenetic silencing of E-cadherin gene of cancer cells was shown to promote the acquisition of invasive phenotype, thereby driving systemic metastasis via EMT (3, 15, 34, 37, 51). These findings suggest the loss of E-cadherin acts as a pleiotropic regulator of cell phenotype, enabling it to function as a master regulator of cell behavior. Therefore, uric acid-induced downregulation of E-cadherin of renal tubular cells may have a critical role in initiating the EMT process and causing renal fibrosis.

Expression of E-cadherin is regulated by complex network in transcription, translation, and posttranslational modification. Several transcription factors have been reported to be involved in
EMT via repression of E-cadherin, which include Snail, Slug, and Twist (4, 5, 36, 44). Previous studies have shown that overexpression of Snail and Slug leads to a reduction of E-cadherin expression (4, 5, 36, 44). Snail and Slug are Snail family of zinc finger transcription factors that have been shown to play a critical role in EMT to determine cancer invasiveness and chemoresponsiveness (1, 48). Similarly, an overexpression of Twist, a transcription factor containing a helix-loop-helix DNA binding domain, also results in decrease of E-cadherin expression (41). In this study, E-cadherin downregulation in uric acid-stimulated NRK cells was associated with a decreased transcription of E-cadherin mRNA and an increased activation of Snail and Slug but not with Twist overexpression. Transforming growth factor-β is reported to downregulate the expression of E-cadherin by inducing Snail, Slug, and Twist in various epithelial cells and cancer cells (7, 53, 58); however, differential regulation of these E-cadherin repressors has been demonstrated in different cells exposed to various stimuli (32, 37, 58).

We also demonstrated enhanced degradation of E-cadherin by uric acid. A decrease in E-cadherin expression in uric acid-stimulated NRK cells was associated with an increase in the degradation of E-cadherin. During EMT, an initial decrease in E-cadherin levels likely occurs by lysosomal degradation (23, 38, 45). Lysosomal degradation was known to be associated with ubiquitination of E-cadherin (45), which was also found in uric acid-stimulated NRK cells in this study. Our data demonstrated uric acid-induced downregulation of E-cadherin mRNA as early as 6 h, followed by E-cadherin ubiquitination and degradation at 24 and 48 h, respectively.

Although EMT of renal tubular cells has been regarded as one of the earliest phenomenon of renal fibrosis, there remain controversies whether EMT is actually a source of interstitial fibroblasts (55, 57). It is mainly due to an insufficient in vivo evidence of EMT (10, 14, 20) and is also related to a misinterpretation of EMT, which can be traced to the belief that an epithelial cell undergoing EMT should become a new fibroblast. However, the definition of EMT does not necessarily require the completion of phenotype transition to a specific cell type. EMT is defined by the process of phenotypic and functional changes that are reminiscent of mesenchymal cells. From this point of view, there is sufficient evidence to assert that at least some EMT markers are expressed during renal fibrosis including in human kidney disease (12, 14, 17, 24, 40, 56). Such biomarkers are very useful compared with conventional histology, because they are sensitive and are expressed at an early stage of the disease. Therefore, uric acid-induced EMT in renal tubules may represent one of the earliest findings in patient with hyperuricemia, which could help in identification of patients at high risk for developing renal fibrosis. Future studies are needed to determine if EMT in the kidneys of hyperuricemic patients could also represent either a therapeutic target or an indicator of therapeutic response.

In conclusion, uric acid, at physiological concentrations, can induce phenotypic transition of renal tubular cells via both a decreased synthesis and an enhanced degradation of E-cadherin, thereby initiating EMT of renal tubules and interstitial fibrosis. In addition to well-recognized mechanisms of uric acid-induced renal disease, including endothelial dysfunction, altered glomerular hemodynamics, and an enhanced local inflammatory reaction in the kidney, uric acid-induced EMT of renal tubular cells may be another mechanism explaining the association of hyperuricemia and progression of renal disease.

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DISCLOSURES

Richard J. Johnson discloses that he has a patent for allopurinol in the treatment of hypertension and also has patent applications related to lowering uric acid as a means for treating diabetes, obesity and diabetic nephropathy. He is also author of the book, _The Fat Switch_ (Mercola.com), which discusses the potential role of fructose and uric acid in the epidemics of obesity, diabetes, and kidney disease.

AUTHOR CONTRIBUTIONS


REFERENCES


