Curcumin Suppresses Epithelial–Mesenchymal Transition of Renal Tubular Epithelial Cells through the Inhibition of Akt/mTOR Pathway

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Curcumin has exhibited a protective effect against development of renal fibrosis in animal models, however, its underlying molecular mechanisms are largely unclear. Therefore, we investigated the anti-fibrotic effects of curcumin in transforming growth factor-β1 (TGF-β1)-induced epithelial-to-mesenchymal transition (EMT), and the mechanism by which it mediates the phosphatidylinositol 3-kinase (PI3K)/Akt pathway. Human kidney tubular epithelial cells (HKCs) were treated with TGF-β1 or curcumin alone, or TGF-β1 in combination with curcumin. The effect of curcumin on cell proliferation was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Expression of E-cadherin, cytokeratin, vimentin, alpha smooth muscle actin (α-SMA), fibroblast-specific protein 1 (FSP1) and key proteins of Akt/mammalian target of rapamycin (mTOR) pathway were analyzed by immunocytochemistry, real-time PCR and Western blot. Low dose curcumin (3.125 and 25 μmol/L) effectively promoted HKC proliferation. When HKCs were co-incubated with TGF-β1 and curcumin for 72 h, curcumin maintained the epithelial morphology in a dose-dependent manner, decreased expression of vimentin, α-SMA and FSP1 normally induced by TGF-β1, and increased expression of E-cadherin, cytokeratin. Importantly, we found that curcumin reduced Akt, mTOR and P70S6K phosphorylation, effectively suppressing the activity of the Akt/mTOR pathway in HKCs. Curcumin also promoted HKC proliferation, and antagonized TGF-β1-driven EMT through the inhibition of Akt/mTOR pathway activity, which may suggest an alternative therapy for renal fibrosis.

Key words renal fibrosis; curcumin; mammalian target of rapamycin (mTOR) pathway; epithelial-to-mesenchymal transition; transforming growth factor-β1 (TGF-β1); renal tubular epithelial cell

Renal fibrosis is a hallmark of end-stage renal diseases and of chronic allograft nephropathy (CAN), irrespective of the nature of initial renal injury.1 Epithelial–mesenchymal transition (EMT) is the key mechanism by which renal fibrosis develops.2,3 Myofibroblasts are considered to be the main effector cells that contribute to the development of progressive renal fibrosis in chronic renal disease. Studies have demonstrated that in kidney fibrosis animal models, about 30% of myofibroblasts are derived via EMT.4,5 Therefore, EMT is the key mechanism by which renal fibrosis develops, and the inhibition of EMT may prevent the progression of fibrosis.

Studies have confirmed that transforming growth factor-β1 (TGF-β1) is a major mediator of renal fibrosis,6,7 and also the major inducer of EMT.1,2 TGF-β1-mediated EMT is thought to be one potential source of the large numbers of myofibroblasts in the fibrotic kidney. TGF-β1 induces EMT of tubular epithelial cells, the loss of epithelial cell markers, such as E-cadherin, and the expression of myofibroblast-specific markers, such as fibroblast-specific protein 1 (FSP1), alpha smooth muscle actin (α-SMA), collagen I, and vimentin. Therefore, a therapeutic strategy to deal with renal fibrosis could be developed from the insights of EMT studies.5

Curcumin, the active component of the traditional Chinese drug Curcuma longa, has anti-oxidative, anti-fibrogenic, anti-inflammatory and anti-proliferative effects.6 Additionally, curcumin has an anti-fibrotic property for which it has been tested as a potential therapeutic agent in a number of pathological conditions, including hepatic, pulmonary and renal fibrosis.7–10 These studies demonstrate that curcumin has the ability to prevent lung, liver and kidney fibrosis in vivo by limiting the accumulation of collagen fibers and extra-cellular matrix (ECM), and by inhibiting fibroblast activation and proliferation.7–10

Although it is well known that TGF-β1 induced EMT by activating the Smad pathway, TGF-β1 can also activate non-Smad-dependent signaling pathway, such as the Akt/mammalian target of rapamycin (mTOR) pathway, which may contribute to renal fibrosis.4,5,11,12 In fact, activation of the protein kinase Akt induces EMT in tubular epithelial cells, as evidenced by down-regulation of numerous epithelial cell specific proteins, including E-cadherin and β-catenin, and up-regulation of the mesenchymal cell specific protein vimentin.13 Several studies have shown that the inhibitor of mTOR, rapamycin, has anti-fibrotic effects, as recently demonstrated in several rat models of chronic kidney disease, including diabetic nephropathy, chronic glomerulosclerosis, and tubulointerstitial fibrosis.14–16 It is thus evident that targeting reductions in mTOR activity attenuates the progression of tubulointerstitial fibrosis in models of diabetic and polycystic kidney disease.14,17–19 Conversely, increased mTOR signaling promotes loss of cadherins and increased fibrosis.14,18,20 Likewise, rapamycin reduced fibrosis and attenuated disease progression in rat models of liver cirrhosis and pulmonary fibrosis.21,22 Therefore, these data suggest that TGF-β1 contributes to EMT by activating the Akt/mTOR pathway, and the inhibition of this pathway can restrain organ fibrosis.
Curcumin is shown to have an antifibrogenic effect, and can prevent fibrosis in the liver and lung, but the underlying mechanisms by which it works remain to be elucidated. Although it is reported that curcumin can block the TGF-β signaling cascade at multiple sites in renal cells, it is still largely unknown whether the Akt/mTOR signaling pathway is involved in the curcumin-induced anti-fibrotic effect. Therefore, we presume that curcumin prevents TGF-β1-induced EMT through the inhibition of the Akt/mTOR signaling pathway.

In this study, we investigated the effects of curcumin on cell viability and the induction of TGF-β1 in tubular epithelial cells, as well as the potential molecular mechanisms by which it acts. Results demonstrated that curcumin could inhibit TGF-β1-induced EMT by interrupting the Akt/mTOR signaling, elucidating a new anti-fibrotic mechanisms applicable in renal fibrosis.

MATERIALS AND METHODS

Materials Curcumin and recombinant human TGF-β1 were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Cell culture reagents and fetal bovine serum (FBS) were purchased from Gibco (Grand Island, NY, U.S.A.). Antibodies against E-cadherin, cytokeratin, vimentin and α-SMA were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.). Antibody against FSP1 was purchased from Abcam (Cambridge, U.K.). Antibodies against Akt, Phosphor-Akt (threonine (Thr)308), mTOR, Phosphor-mTOR (serine (Ser)2481), Phosphor-p70S6K (Thr421/Ser424), Phosphor-4E-Akt (threonine (Thr)308), mTOR, Phosphor-mTOR, and Phosphor-eIF4E were purchased from Cell Signaling Technology (Beverly, MA, U.S.A.).

3-(4,5-Dimethylthiazol-2-yI)-2,5-diphenyltetrazolium Bromide (MTT) Assay to Determine Cell Viability The human renal proximal tubular cell line, HKC, was purchased from American Type Culture Collection (ATCC) and maintained in Dulbecco’s modified Eagle’s medium (DMEM, Gibco) supplemented with 10% FBS, Gibco. The proliferation of HKCs was determined by MTT (Sigma-Aldrich) assay. Cells were then treated with curcumin (0.78, 1.56, 3.125, 6.25, 12.5, 25, 50 and 100 µmol/L) or TGF-β1 (0, 1, 5, 10ng/mL) alone for 72 h, respectively. Subsequently, in another set of experiments, HKCs treated with 5ng/mL TGF-β1 were simultaneously exposed to 3.125, 6.25, 12.5, 25 and 100 µmol/L curcumin for 72 h. Following treatment, 20µL of MTT stock solution (5mg/mL) was added to each well and incubated for 4h. The supernatant was removed, and cells were lyzed completely in 100µL dimethyl sulfoxide (DMSO). The absorbance was detected by microplate reader at 490 nm. The assay was performed in five independent experiments.

Morphological Assessment of HKCs The cells were grown in 6-well plates, and then treated with or without (the control) TGF-β1 alone or in combination with curcumin (6.25, 12.5, 25, 50, 100 µmol/L) for 72 h. The morphological changes of HKCs were observed under an inverted phase contrast microscope.

Immunohistochemical Staining for FSP1 The cells were treated with different concentrations of TGF-β1 (0, 1, 5, 10ng/mL) for 72 h. Cultured cells were then fixed with 4% paraformaldehyde in phosphate buffered saline (PBS) for 10 min at room temperature and permeabilized with 0.2% Triton X-100 (Sigma-Aldrich) for 5 min. Cells were then incubated with blocking solution consisting of PBS and 10% goat serum at room temperature for 30 min. Endogenous peroxide activity was revealed with 0.3% hydrogen peroxide solution. Slides were then incubated sequentially with primary antibody at 4°C overnight. Primary antibody against FSP1 (1:200, Abcam) was applied and followed by biotinylated anti-rabbit antibody. After washing three times, slides were incubated with an avidin–biotin conjugate of horseradish peroxidase in PBS for 30 min at room temperature. The specimens were developed with dianaminobenzidine (DAB). The photos were taken by randomly selecting 5 high-power fields (×400). We used the image analysis software Image Propus 5.0 to calculate the total cells number and FSP1-positive cells number (brown) of 5 hpf, and the percentage of FSP1-positive cells/the total cells was calculated. Experiments were repeated at least three times.

Quantitative Reverse-Transcription (RT)-PCR Quantitative RT-PCR analysis was performed to detect E-cadherin, cytokeratin, vimentin and α-SMA mRNA expression. Total RNA was extracted from cells at the indicated times using the RNeasy mini kit (Qiagen, Hombrechtikon, Switzerland) and treated with deoxyribonuclease (DNase) I (Promega Corp., U.S.A.), according to the manufacturer’s instructions. cDNA was produced using the GeneAmp RNA PCR kit (Applied Biosystems, Rotkreuz, Switzerland). Quantitative PCR analysis was performed with Power SYBR Green PCR master mix (Applied Biosystems) using commercially available primers (QuantiTect Primer, Qiagen) and using β-actin as an endogenous control. Reactions were run on iQ5 Cycler thermocycler (Bio-Rad). Results represent the average obtained from at least three separate experiments.

Western Blot Analysis For Western blot analysis, treated cells were harvested at the indicated times by the addition of ice-cold lysis buffer for 15 min. The homogenate was centrifuged for 10 min at 12000 rpm and 4°C. Protein concentration was determined using the Bradford method. Equal amounts of cell extract were subject to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes. After blocking with 5% non-fat milk in TBST for 60 min, the membranes were incubated overnight at 4°C with the following primary antibodies: E-cadherin, cytokeratin, vimentin, α-SMA, anti-Akt, anti-mTOR, anti-Phosphor-Akt, anti-Phosphor-mTOR, anti-Phosphor-p70S6K, anti-Phosphor-4E-BP1, anti-Phosphor-eIF4E. Lastly, the membranes were incubated with a horseradish peroxidase (HRP)-conjugated secondary antibody for 60 min at 37°C, which were then detected by an ECL luminescent detection system. Band density was analyzed using LabWorks 4.6 analysis software.

Statistical Analysis Values are expressed as the mean±standard deviation (S.D.). Statistical analyses were performed using SPSS software. Student’s t-test was used to analyze the statistical significance in the differences between two groups. The multiple comparison was analyzed with a Tukey test. For all comparisons, a p-value less than 0.05 was considered statistically significant.

RESULTS

Cell Viability Analysis Expression of TGF-β1 is el-

evated in multiple forms of experimental and human kidney disease, and it is a central mediator in renal fibrosis progression. TGF-β1 is a powerful suppressor of cell growth and proliferation, particularly in cells of epithelial, and endothelial lineage. Treatment with TGF-β1 at concentrations of more than 5 ng/mL significantly inhibited the proliferation of HKCs in a dose-dependent manner (Fig. 1a). However, we also found that low concentration (3.125–25 μmol/L) curcumin significantly enhanced the proliferation of HKCs, and a high concentration (100 μmol/L) of curcumin decreased HKC proliferation (Fig. 1b). When HKCs were treated with 5 ng/mL TGF-β1 in combination with various concentrations of curcumin, curcumin effectively antagonized TGF-β1-induced inhibition of HKC proliferation at doses between 12.5–50 μmol/L (Fig. 1c). Collectively, these data showed that low concentration curcumin significantly promoted the growth and proliferation of the renal tubular epithelial cells, while high concentration curcumin is cytotoxic. Low and moderate doses of curcumin have the potential to antagonize the suppressive effects of TGF-β1 on renal tubular epithelial cell proliferation.

**TGF-β1 Induces EMT of HKCs** HKCs cultured in the absence of TGF-β1 showed classical epithelial cobblestone morphology. After TGF-β1 treatment for 72 h, cells acquired a spindle-shaped morphology with disruption of cell–cell contacts, resembling fibroblast-like morphology. With the increase in TGF-β1 concentration, profound morphologic changes occurred, with cells developing marked hypertrophy, becoming elongated, and losing the cobblestone growth pattern (Fig. 2a). Concomitantly with the change in morphology, the phenotype markers of these cells changed. The expression of epithelial markers E-cadherin and cytokeratin was remarkably reduced, while the expression of mesenchymal markers, α-SMA and vimentin was remarkably upregulated (Figs. 2b, c). In addition, immunostaining showed that TGF-β1 treatment significantly increased the number of FSP1-expressing myofibroblasts in cultured HKCs in a dose-dependent manner (Figs. 2d, e). This data suggested that the renal tubular epithelial cells underwent morphological and phenotypic marker changes upon exposure to TGF-β1, transdifferentiating into myofibroblasts.

**Preventive Effects of Curcumin on TGF-β1-Induced EMT in HKCs** Curcumin has been proposed as a potential anti-fibrotic agent. In order to investigate the anti-fibrotic effects of curcumin on TGF-β1-induced EMT, HKCs were incubated with culture medium containing TGF-β1 or TGF-β1 combined with various dose of curcumin for 72 h. Then cell morphology were observed under phase contrast light microscopy. As shown in Fig. 3a, HKC cells exhibited the typical cobblestone morphology of epithelial cells when grown in culture. After TGF-β1 treatment for 72 h, they became elongated, disassociated from neighboring cells, and lost their cobblestone monolayer pattern. The simultaneous incubation of TGF-β1 with different concentrations curcumin (12.5, 25, and 50 μmol/L) protected the epithelial morphology of HKCs from TGF-β1-induced EMT, HKCs cultured in the absence of TGF-β1 for 72 h induced up-regulation of vimentin and α-SMA protein and mRNA levels, and down-regulation of E-cadherin and cytokeratin expression. However, the expression of vimentin and α-SMA was remarkably inhibited, while the expression of E-cadherin and cytokeratin was promoted in HKCs treated with TGF-β1 and curcumin. Further, immunostaining showed that TGF-β1 treatment significantly increased the number of FSP1-positive myofibroblasts in cultured HKCs, however, the number of FSP1-positive cells was significantly reduced when HKCs were incubated in culture medium containing TGF-β1 combined with 12.5–100μmol/L curcumin (Figs. 3f, g). These results indicated that curcumin not only prevented the de novo expression of the myofibroblast markers vimentin, α-SMA and FSP1, but also inhibited the loss of the epithelial markers E-cadherin and cytokeratin in HKCs, indicating that curcumin could prevent TGF-β1-induced EMT in HKCs.

**Curcumin Prevents TGF-β1-Induced EMT through Inhibition of Akt/mTOR Signaling Pathway in HKCs** Some studies have shown that curcumin has a significant inhibitory effect on fibrosis in pulmonary, hepatic and renal fibrosis in rat models. Likewise, our results demonstrated that curcumin can antagonize TGF-β1-induced EMT in vitro. However, the molecular mechanisms by which curcumin exerts anti-fibrosis effects is not clear. It is reported that the Akt/mTOR signaling pathway is closely associated with renal tubulointerstitial fibrosis. Reduction in mTOR activity attenuates the progression of
tubulointerstitial fibrosis in models of diabetic and polycystic kidney disease. The evidences in carcinogenic models further suggest that increased mTOR signaling promotes loss of cadherins and increased fibrosis. Accordingly, we hypothesized that TGF-β1 contributes to EMT at least in part through the activation of the mTOR pathway, and the anti-fibrotic effects of curcumin may be mediated partially through the attenuation of Akt/mTOR signaling, thus antagonizing TGF-β1-induced EMT.

As Fig. 4 shows, after the HKCs were treated with TGF-β1 alone, no changes in total Akt and mTOR protein levels, and significantly increased phosphorylated Akt and mTOR protein levels, were observed. Treatment with both TGF-β1 and curcumin resulted in significantly decreased Akt and mTOR phosphorylation in a curcumin dose-dependent manner (Figs. 4a, b). Likewise, TGF-β1 increased the phosphorylation of downstream targets of the Akt/mTOR pathway, such as p70S6K, 4E-BP1 and eIF4E. However, the phosphorylation of these proteins was significantly decreased in HKCs after co-treatment with curcumin, (Figs. 4c, d). These results demonstrated that curcumin antagonized TGF-β1-induced EMT at least in part through the inhibition of the Akt/mTOR pathway. This elucidates one of the molecule mechanisms by which curcumin exerts its anti-fibrosis effect.

DISCUSSION

Renal fibrosis/tubulointerstitial fibrosis is the mutual, terminal fate of many chronic renal diseases and kidney transplantation. In progressive states, many factors have been reported as fibrogenic in the kidney. TGF-β1 is known as a key profibrotic mediator in development of tubulointerstitial fibrosis.
TGF-β1 induces tubular epithelial-to-mesenchymal transition (EMT), which is thought to be one of the major events in the pathogenesis of renal fibrosis.\textsuperscript{4,25,26} In this study, we analyzed the anti-fibrotic properties of curcumin in renal cells, and discovered that these anti-fibrotic properties are mediated through the inhibition of TGF-β1-induced EMT. Furthermore, we discovered that the inhibition of TGF-β1-induced EMT is via the inhibition of Akt/mTOR pathway activity.

In this study, HKCs exposed to various concentrations of TGF-β1 for 72 h, underwent a complete transition from epithelial cells to myofibroblasts. The typical cobblestone morphology of epithelial cells was converted to the spindle-like morphology of myofibroblasts. This transition was accompanied by the down-regulation of epithelial markers E-cadherin and cytokeratin, and up-regulation of stroma cell markers vimentin and α-SMA, in a dose-dependent manner. Additionally, TGF-β1 inhibited the proliferation of HKCs in a dose-dependent manner. Therefore, TGF-β1 is the most potent inducer that is capable of initiating and completing the entire EMT course, which is consistent with the previous report.\textsuperscript{5,26,27}

Curcumin is a naturally occurring polyphenolic compound derived from the root of \textit{Curcuma longa}, and is consumed...
Curcumin's multiple pharmacological activities, like its antioxidative effects, anti-inflammatory effects and anti-fibrogenic effects, have been well demonstrated. The anti-fibrotic effects of curcumin were first demonstrated in the bleomycin model of pulmonary fibrosis, where curcumin application prevented collagen accumulation. Sequentially, curcumin was shown to have the ability to prevent kidney fibrosis in vivo by limiting the accumulation of collagen fibers. As TGF-β-induced EMT is the most important pathogenesis leading to kidney fibrosis, we firstly observed the influence of curcumin on the transdifferentiation of renal tubular epithelial cells. As evidenced by restoration of epithelial morphology, an increased in E-cadherin and cytokeratin expression, and decreased α-SMA and vimentin expression, our results demonstrated that curcumin exhibits an inhibitory effect on TGF-β1-induced EMT at concentrations between 12.5–100 μmol/L. Moreover, this data strongly suggests that curcumin has the ability to maintain the normal phenotype and function of tubular epithelium.

Numerous therapeutic effects of curcumin have been confirmed by modern scientific research. A great majority of these studies principally focused on the cytotoxic activity (anti-proliferation and pro-apoptosis) of curcumin against a range of human tumor cell lines in vitro and in tumor animal models. They showed that curcumin induced apoptosis in tumor cell lines cells (curcumin-sensitive) in a dose-dependent and time-dependent manner. Moreover, the apoptosis was mediated through the generation of reactive oxygen species (ROS) production at high concentrations of curcumin trigger the inhibition of cell growth and apoptosis. Therefore, curcumin has no effect on non-transformed cell lines and normal cells, which shows neither superoxide generation nor the induction of a stress response when exposed to curcumin. Therefore, curcumin has been shown to selectively kill tumor cells and abnormal cells, but not cause any damage to normal cells. In fact, a study found that curcumin exerted biphasic effects on cultured multi-potent neural progenitor cells, low concentrations stimulated cell proliferation, whereas high concentrations were cytotoxic. Therefore, curcumin on cell proliferation was mediated through activation of extracellular signal-regulated kinases (ERKs) and p38 kinases. Our results are consisted with these reports. Our results also show that low dose curcumin promotes the proliferation of renal tubule epithelial cells at concentrations of 3.125–25 μmol/L, and inhibits HKCs proliferation at 100 μmol/L.

Furthermore, we found that after co-treatment with TGF-β1 and curcumin, not only the cell morphology of HKCs was maintained, but the density of HKCs under phase contrast light microscopy also increased at curcumin concentrations of 12.5–50 μmol/L. We presumed that curcumin could also antagonize the inhibition effect of TGF-β1 on the proliferation of HKCs. Indeed, as we had expected, co-administration of TGF-β1 with curcumin promoted the proliferation of HKCs at curcumin concentrations of 12.5–50 μmol/L. The activation of ERK and p38 MAP kinases mediate the proliferative effects of low dose curcumin on normal cells. The reduction of reactive oxygen species (ROS) production at low concentrations and induction of ROS production at high concentrations of curcumin trigger the inhibition of cell growth and apoptosis. Therefore, by gradually increasing the concentration of curcumin, the pro-proliferation effects disappear, and cell growth suppressive effects, via intracellular ROS generation, appear. Alternatively, curcumin could down-regulate TGF-β1 receptor type II (TβRII) in renal cells, thereby, re-
duc ing TGF-β1-induced expression of pro-fibrotic genes and proteins. More, importantly, this maybe abolish the potent anti-mitogenic and pro-apoptotic effects of TGF-β1 which is mediated through TβRII mediated Smad pathway, thus allowing HKC escape from Smad-induced growth inhibition.33,34 Therefore, promotion of kidney tubular epithelial cells proliferation by curcumin provides for an attractive explanation for the anti-fibrotic effects of curcumin observed in vivo in fibrotic kidney disease. Moreover, in vivo studies also found that a higher dose of curcumin fails to increase the numbers of BrdU-positive cells and were not toxic to cells in the adult hippocampus. Conversely, a much lower dose of curcumin significantly increased the proliferation of the cells.31 Together, this data suggests that the biological activity of curcumin is closely associated with the dose of curcumin treatment in vivo and in vitro, and the cell type.

Although EMT and fibrosis is classically driven by TGF-β1-induced activation of the Smad-dependent signaling pathway,5,22,26 TGF-β1 can also activate non-Smad-dependent signaling events that may contribute to fibrosis, such as Akt/mTOR, p38 mitogen-activated protein kinase (MAPK), integrin-linked kinase (ILK), RhoA, and β-catenin pathway.11,35-37 mTOR is a Ser/Thr kinase that regulates transcription and protein synthesis, cell growth, proliferation, cell migration and cell phenotype transition.5,11 Several studies have demonstrated that the inhibition of the mTOR pathway by rapamycin, can restrain organ fibrosis,13-20 reduce the expression of fibrosis-associated genes, and contribute to the inhibition of the progression of tubulointerstitial fibrosis in several rat models of chronic kidney disease.38,39 A study demonstrated that TGF-β1 induces epithelial to mesenchymal transition (EMT) through the activation of PI3K/Akt/mTOR pathway,24 therefore, rapamycin could prevent TGF-β1-induced transition of human renal tubular epithelial cells to myofibroblasts, and reverse myofibroblast cell morphology to tubular epithelial morphology.40 Similarly, our results found that curcumin antagonized TGF-β1-induced EMT comitant with reduced phosphorylation of Akt, mTOR and their downstream key proteins p70S6K and 4E-BP1, which lead to dampening of the Akt/mTOR pathway. Therefore, we suggested that the anti-fibrotic effects of curcumin may be mediated through the inhibition of the Akt/mTOR pathway, and that curcumin might be an effective antifibrotic drug in the treatment of chronic kidney disease.

In conclusion, our results confirmed that low dose curcumin could effectively promote renal tubular epithelial cell proliferation. Importantly, curcumin possesses a remarkable ability to block tubular EMT induced by TGF-β1, and maintain the morphology and phenotype of renal tubule epithelial cells. We also demonstrated that curcumin blocked TGF-β1-induced EMT at least partially through the inhibition of the Akt/mTOR pathway, which may be one mechanism by which curcumin exercises its anti-fibrotic effects. Lastly, there are almost no side effects associated with curcumin use, even at high doses of oral administration.41 Therefore, curcumin may be a promising anti-fibrotic agent for therapy of renal interstitial fibrosis in patients with chronic renal diseases and chronic allograft nephropathy (CAN). The progression of these kidney disease can be slowed down and a better long-term allograft survival can be expected.

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Conflict of Interest The authors declare no conflict of interest

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