

# Allicin inhibits tubular epithelial-myofibroblast transdifferentiation under high glucose conditions *in vitro*

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**Abstract.** Previous studies have suggested that tubular epithelial-mesenchymal transition (EMT) is an important event in renal tubulointerstitial fibrosis, which is a clinical characteristic of diabetic nephropathy. The present study aimed to investigate the effect of allicin, the major biological active component of garlic, on the EMT of a human renal proximal tubular epithelial cell line (HK-2) cultured under high glucose concentrations. HK-2 cells were exposed for 48 h to 5.5 or 25 mmol/l D-glucose, 25 mmol/l D-glucose plus allicin (2.5, 5, 10 or 20  $\mu$ g/ml) or 25 mmol/l D-glucose plus 20  $\mu$ mol/l PD98059, a selective inhibitor of the mitogen activated protein kinase/extracellular signal-regulated kinase (ERK) signaling pathway. The EMT of HK-2 cells was assessed by analyzing the protein expression of E-cadherin,  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA), vimentin and collagen I via immunocytochemistry. In addition, reverse transcription-quantitative polymerase chain reaction and western blotting were used to detect the expression levels of transforming growth factor (TGF)- $\beta$ 1 and phosphorylated (p)-ERK1/2. Marked morphological changes were observed in HK-2 cells cultured under high glucose conditions, and these changes were abrogated by simultaneous incubation with allicin and PD98059. The expression levels of  $\alpha$ -SMA, vimentin and collagen I were significantly increased in HK-2 cells cultured under high glucose conditions, as compared with those cultured under normal glucose conditions ( $P < 0.01$ ). Conversely, the expression levels of E-cadherin were significantly decreased upon stimulation with high glucose ( $P < 0.01$ ). Furthermore, the expression levels of TGF- $\beta$ 1 and p-ERK1/2 were significantly upregulated in HK-2 cells cultured under high glucose conditions, as compared with those cultured under normal glucose conditions ( $P < 0.05$ ).

Allicin partially reversed the high-glucose-induced increase in  $\alpha$ -SMA, vimentin and collagen I expression ( $P < 0.01$  at 20  $\mu$ g/ml), increased the expression of E-cadherin, and significantly downregulated the high glucose-induced expression of TGF- $\beta$ 1 and p-ERK1/2 in a dose-dependent manner ( $P < 0.05$ ). The results of the present study suggested that high glucose concentrations induced the EMT of HK-2 cells, and that allicin was able to inhibit the EMT, potentially via regulation of the ERK1/2-TGF- $\beta$ 1 signaling pathway.

## Introduction

Tubulointerstitial fibrosis, which is considered the final common pathway of progressive kidney diseases and eventually leads to end-stage renal disease, is a result of the tubular epithelial-to-mesenchymal transition (EMT) and excessive accumulation of extracellular matrix (ECM) that characterize the majority of chronic kidney diseases, including diabetic nephropathy (DN) (1). Previous studies have demonstrated that high glucose conditions induce the EMT in renal proximal tubular cells *in vitro* and *in vivo* (2-5). In addition, it has been reported that myofibroblast formation is a critical step in the pathogenesis of tubulointerstitial fibrosis, and it has been shown to be a key step in DN progression (5,6). Myofibroblasts, which are considered to be one of the principle effective cells derived from the renal tubular EMT, are responsible for the ECM and have a central role in progressive renal fibrosis (7,8). In the process of the EMT, renal proximal tubular cells have been shown to contribute to renal interstitial fibrosis; the cells lose their epithelial phenotype and acquire a myofibroblastic phenotype, which is characterized by an increased motility, extracellular protein synthesis and invasiveness (9). Irrespective of the initial causes, interstitial fibrosis is a remarkable process that is characterized by *de novo* activation of the mesenchymal markers,  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) and vimentin, and the excessive deposition of ECM components in the tubulointerstitium under pathological conditions by the myofibroblasts (8,10,11). Therefore, it is important to investigate the molecular mechanisms underlying tubulointerstitial fibrosis in order to identify novel targets for the effective treatment, prevention and delay of DN.

The EMT is regulated by numerous growth factors and hormones (12). Transforming growth factor (TGF)- $\beta$ 1 has been shown to be a potent growth factor that has a pivotal role

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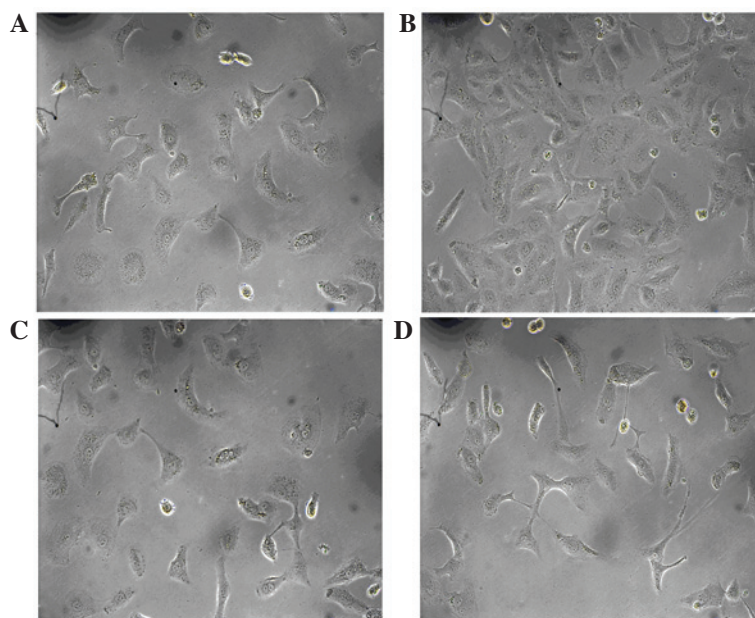


Figure 2. Effect of allixin on high glucose-induced morphological changes in HK-2 cells at 48 h. Phase-contrast microscopy images are presented, showing the morphology of HK-2 cells following exposure to (A) normal glucose (control), (B) high glucose, (C) high glucose plus 20  $\mu\text{g/ml}$  allixin or (D) high glucose plus 20  $\mu\text{mol/l}$  PD98059. (magnification, x200).

ThermoScript™ RT-PCR System (Thermo Fisher Scientific, Inc.), after which qPCR was performed using the SYBR Premix Ex Taq kit and PCR primers on an ABI Prism 7500 thermal cycler (Applied Biosystems; Thermo Fisher Scientific, Inc.), according to manufacturer's protocol. The PCR cycling conditions were as follows: 95°C for 5 min, followed by 40 cycles at 95°C for 15 sec, 60°C for 20 sec and 72°C for 20 sec, and a final extension at 72°C for 10 min. The primer sequences were: TGF- $\beta$ 1 (362 bp) forward, 5'-ACTACGCCA AAG AAGTCACCC-3' and reverse, 5'-AAGCCCTGTATT CCGTCTCC-3'; and  $\beta$ -actin (317 bp) forward, 5'-CGTACC ACTGGCATTGTGAT-3' and reverse, 5'-TTGCCGATA GTGATGACCTG-3'. Reaction specificity was confirmed by agarose gel electrophoresis analysis of PCR products. Ratios for TGF- $\beta$ 1/ $\beta$ -actin mRNA were calculated for each sample and are expressed as the mean  $\pm$  standard error of the mean. Each sample was run in triplicate. The expression of each gene was normalized against that of  $\beta$ -actin. The relative quantity of mRNA was calculated using the  $2^{-\Delta\Delta C_q}$  method (39).

**Western blotting.** HK-2 cells were plated in 10-cm culture plates with or without stimuli and various treatments: HK-2 cells were passaged until 80% confluent. Subconfluent cells were incubated with serum-free medium for 24 h and divided into four groups, as follows: i) Normal glucose group (5.5 mmol/l; control group); ii) high glucose group (25 mmol/l); iii) high glucose (25 mmol/l) and allixin (2.5, 5, 10 or 20  $\mu\text{g/ml}$ ) group; and iv) high glucose (25 mmol/l) and PD98059 (20  $\mu\text{mol/l}$ ) group. Following treatment, the cells were incubated for 48 h. The cells were then analyzed by western blotting, as described previously (40). Cells were collected and lysed using lysis buffer [20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1% Triton, 1% NP-40, 2.5 mM sodium pyrophosphate, 1 mM  $\beta$ -glycerophosphate, 1 mM leupeptin, 1 mM phenylmethylsulfonyl fluoride], and samples were centrifuged at

12,000  $\times$  g for 30 min at 4°C. The concentration of protein in each cell lysate was determined using a BCA Protein Assay kit (Pierce; Thermo Fisher Scientific, Inc.). Equal quantities of cell protein lysates (20  $\mu\text{g}$ ) were mixed with 2X sodium dodecyl sulfate (SDS) loading buffer containing dithiothreitol and heated at 100°C for 10 min, prior to separation by 10% SDS-PAGE. Subsequently, the proteins were transferred to a polyvinylidene difluoride membrane and non-specific binding was blocked with 5% non-fat dry milk in phosphate-buffered saline containing 0.02% v/v Tween-20. The membrane was incubated overnight at 4°C with one of the following primary antibodies: Rabbit anti-p-ERK1/2 (1:500), anti-ERK1/2 (1:500) and anti-TGF- $\beta$ 1 (1:500) polyclonal antibodies, and mouse anti- $\beta$ -actin monoclonal antibody (1:5,000). After three washes with Tris-buffered saline with Tween 20, the membranes were incubated for 2 h at room temperature with HRP-conjugated anti-rabbit or anti-mouse IgG (1:10,000). After further washing, the membrane was detected with ECL chemiluminescence, and band intensities were quantified by densitometry using Image Lab software (Bio-Rad Laboratories, Inc., Hercules, CA, USA).  $\beta$ -actin was used as a loading control.

**Immunocytochemistry.** HK-2 cells in the various groups were analyzed for tubular EMT using microwave-based two-color immunostaining. Briefly, cells were fixed in 4% paraformaldehyde and pre-incubated with 10% FBS and 10% normal goat serum (Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China) to block non-specific binding. Subsequently, the cells were incubated with rabbit anti- $\alpha$ -SMA, rabbit anti-vimentin, mouse anti-E-cadherin and mouse anti-collagen I monoclonal antibodies or an isotype control IgG at 4°C overnight. Following inactivation of endogenous peroxidase activity, the cells were incubated with HRP-conjugated goat anti-rabbit or goat anti-mouse IgG, then by rabbit or mouse anti-peroxidase complexes. Slides

were then developed with 3,3'-diaminobenzidine to produce a brown product. Finally, all sections were counterstained with hematoxylin and mounted on cover-slips using aqueous mounting medium. All procedures were performed at room temperature. Brown-yellow granules, as assessed by light microscopy, were regarded as positive cells. Images were analyzed using Image-Pro Plus 6.0 image analysis software (Media Cybernetics, Inc., Rockville, MD, USA). The stained field sections were then assessed for morphological changes using a light microscope at x400 magnification. For all groups, sections were taken from the same region. An average gray-scale value represented the measurement value. The gray-scale value of positive protein expression was determined and statistically analyzed.

**Fluorescence immunocytochemistry.** HK-2 cells were cultured in DMEM containing 5.5 mmol/l D-glucose. Upon reaching 80% confluence, cells were synchronized with FBS-free medium (5.5 mmol/l D-glucose) for 24 h, then cultured with or without various treatments for 48 h. Subconfluent cells were incubated with serum-free medium for 24 h and divided into four groups, as follows: i) Normal glucose (5.5 mmol/l; control group); ii) high glucose group (25 mmol/l); iii) high glucose (25 mmol/l) plus allicin (2.5, 5, 10 or 20  $\mu\text{g/ml}$ ) group; and iv) high glucose (25 mmol/l) plus PD98059 (20  $\mu\text{mol/l}$ ) group. Following treatment, the cells were incubated for 48 h. Cells were then fixed in 4% paraformaldehyde for 30 min, permeabilized with 0.1% Triton X-100 for 15 min and incubated with 10% normal goat serum blocking buffer for 1 h at 37°C. Subsequently, the cells were incubated overnight at 4°C with rabbit anti- $\alpha$ -SMA (1:200), rabbit anti-vimentin (1:200), mouse anti-E-cadherin (1:200) and mouse anti-collagen I (1:100) monoclonal antibodies. Cells were then incubated with FITC-conjugated secondary antibody (1:200) for 1 h at 37°C in the dark, then stained with propidium iodide for 1 h. The negative control consisted of cells incubated with IgG instead of primary antibody. Cells were visualized and photographed using a laser scanning confocal microscope (Olympus Corp., Tokyo, Japan). Olympus FluoView (Olympus Corp.) and Velocity 4.1 (Improvision; Velocity Software Inc, Mountain View, CA, USA) software were used for image processing, deconvolution, and quantitative imaging analyses wherever appropriate. Confocal images acquired under the identical exposure time and instrument settings among different groups were used for colocalization and quantitative fluorescence intensity analyses.

**Statistical analysis.** Data are expressed as the mean  $\pm$  standard error of the mean. Statistical significance was determined using one-way analysis of variance followed by Fisher's least significant difference test. Statistical analyses were performed using SPSS 16.0 software for Windows (SPSS, Inc., Chicago, IL, USA).  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Effect of allicin on high glucose-induced morphological changes in HK-2 cells.** To assess the effect of allicin on cell morphology, HK-2 cells were serum deprived for 24 h and

exposed to high glucose conditions for 48 h, after which the cells were observed by inverted phase-contrast microscopy. The normal group had the typical epithelial cuboidal shape, with a cobblestone morphology (Fig. 2A). Conversely, cells in the high glucose group exhibited an elongated, fibroblast-like phenotype (Fig. 2B). Simultaneous incubation with allicin (20  $\mu\text{g/ml}$ ) or PD98059 (20  $\mu\text{g/ml}$ ) prevented the high glucose-induced morphological changes in the majority of cells, with cells retaining epithelial polarity and a cobblestone growth pattern, in the absence of hypertrophy and an elongated morphology (Fig. 2C and D).

**Effect of allicin on the expression levels of E-cadherin,  $\alpha$ -SMA, vimentin and collagen I in HK-2 cells cultured under high glucose conditions.** To confirm the transformation of HK-2 cells into a fibroblast-like phenotype, the expression levels of the epithelial marker, E-cadherin, and the mesenchymal markers,  $\alpha$ -SMA and vimentin, were determined by immunohistochemistry and fluorescence immunocytochemistry. In addition, the expression levels of collagen I, an important component of the ECM, were also evaluated. The expression levels of  $\alpha$ -SMA, vimentin and collagen I were significantly increased and peaked at 48 h in the high glucose group, as compared with the control group ( $P < 0.01$ ; Figs. 3 and 4). Conversely, the expression levels of E-cadherin were markedly decreased in the high glucose group, as compared with the control group ( $P < 0.01$ ; Figs. 3 and 4). Allicin reversed the high glucose-induced changes at 48 h in a dose-dependent manner, with the difference being significant at 20  $\mu\text{g/ml}$  allicin ( $P < 0.01$  vs. the high glucose group). Upon incubation with PD98059 for 48 h, the expression levels of  $\alpha$ -SMA, vimentin and collagen I were markedly decreased and those of E-cadherin were markedly increased, as compared with those of the high glucose cells ( $P < 0.01$ ), although they were not significantly different from the control cells ( $P > 0.05$ ).

**Effect of allicin on the expression level of TGF- $\beta$ 1 in HK-2 cells cultured under high glucose conditions.** The mRNA and protein expression levels of TGF- $\beta$ 1 were measured by RT-qPCR and western blotting, respectively (Fig. 5). RT-qPCR demonstrated that the mRNA expression levels of TGF- $\beta$ 1 were significantly increased at 48 h in the high glucose group, as compared with the control group ( $P < 0.05$ ). Allicin treatment resulted in a dose-dependent decrease in the mRNA expression levels of TGF- $\beta$ 1 at 48 h; in particular the differences were significant at 10 and 20  $\mu\text{g/ml}$  allicin ( $P < 0.05$  vs. the high glucose group). Upon intervention with PD98059, the mRNA expression levels of TGF- $\beta$ 1 were significantly reduced, as compared with the high glucose group ( $P < 0.05$ ), although they were increased, as compared with the normal control cells ( $P < 0.05$ ). These results were consistent with the results of the western blot analysis. The protein expression levels of TGF- $\beta$ 1 were significantly increased and peaked at 48 h in the high glucose group, as compared with the control group ( $P < 0.05$ ). Allicin decreased the protein expression levels of TGF- $\beta$ 1 at 48 h in a dose-dependent manner, in particular at 20  $\mu\text{g/ml}$  where the inhibition rate was 55.7%, as compared with the high glucose group ( $P < 0.05$ ). Upon intervention with PD98059, the protein expression levels of TGF- $\beta$ 1 were also significantly reduced,

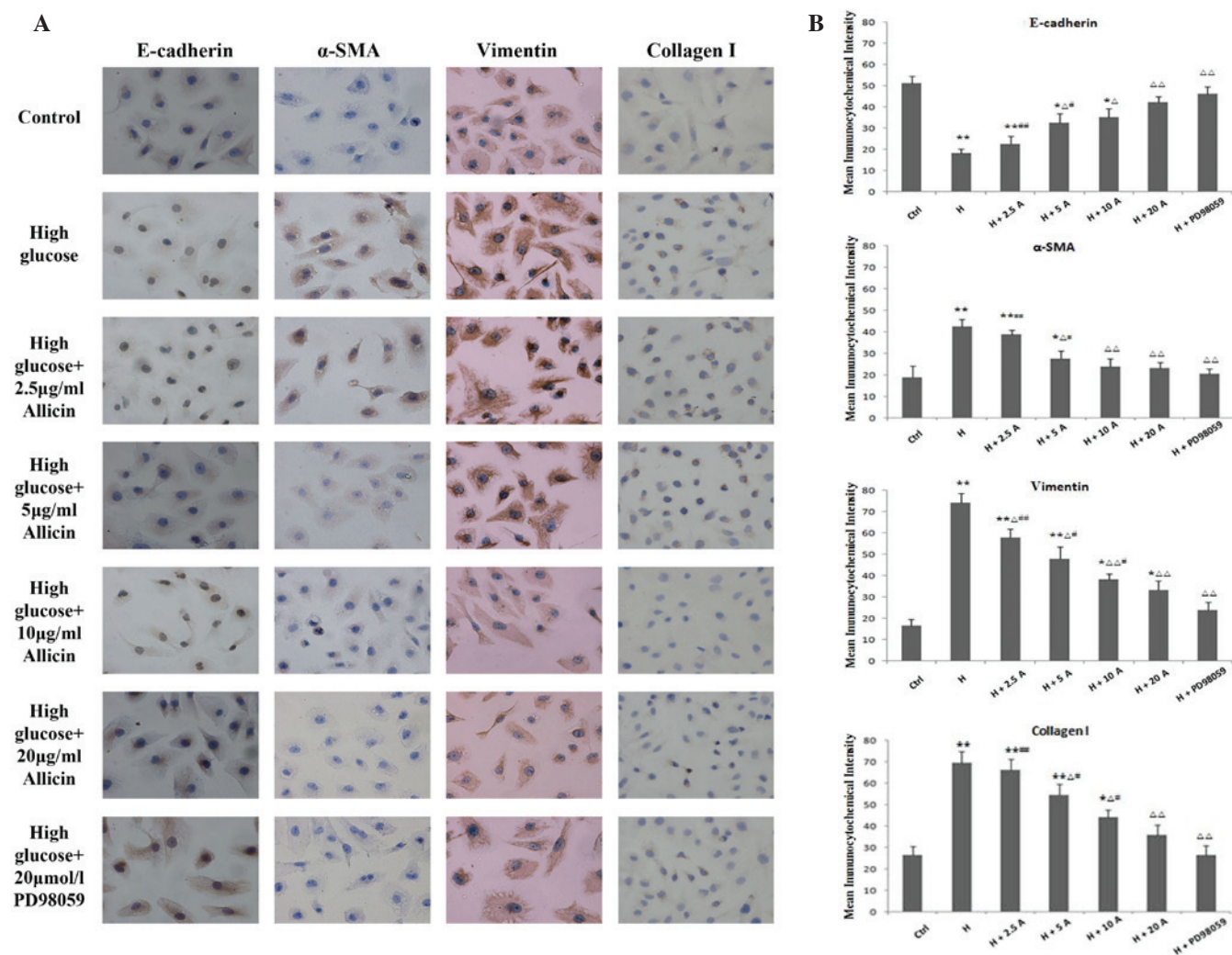


Figure 3. (A) Immunocytochemical staining of E-cadherin,  $\alpha$ -SMA, vimentin and collagen I in HK-2 cells cultured for 48 h (magnification,  $\times 200$ ). (B) HK-2 cells were exposed to normal glucose (ctrl), high glucose, high glucose plus 2.5, 5, 10 and 20  $\mu\text{g/ml}$  allicin or high glucose plus 20  $\mu\text{mol/l}$  PD98059. \* $P < 0.05$  and \*\* $P < 0.01$  vs. Ctrl;  $^{\Delta}P < 0.05$  and  $^{\Delta\Delta}P < 0.01$  vs. H;  $^{\#}P < 0.05$  and  $^{\#\#}P < 0.01$  vs. H + PD98059.  $\alpha$ -SMA,  $\alpha$ -smooth muscle actin; Ctrl, control; H, high glucose; A, allicin.

as compared with the high glucose group ( $P < 0.05$ ), and were not significantly different, as compared with the control group ( $P > 0.05$ ).

*Effect of allicin on p-ERK1/2 expression in HK-2 cells cultured under high glucose conditions.* To further elucidate the molecular mechanisms underlying the allicin-mediated inhibition of the EMT process in HK-2 cells cultured under high glucose conditions, the potential involvement of the ERK1/2 signaling pathway was investigated by western blotting. The levels of p-ERK1/2 were significantly increased in the high glucose group at 48 h, as compared with the control group ( $P < 0.05$ ; Fig. 6). However, high glucose-induced ERK1/2 phosphorylation was significantly attenuated by pre-treatment with PD98059 ( $P < 0.05$ ; Fig. 6), a specific inhibitor of the MAPK/ERK kinase, which is the upstream activator of ERK1/2. Similarly, treatment with allicin significantly decreased the protein expression levels of p-ERK1/2 in a dose-dependent manner, in particular at 20  $\mu\text{g/ml}$ , with inhibition rates of 37.7%, as compared with the high glucose group ( $P < 0.05$ ). However, the levels were significantly increased, as compared with the control group ( $P < 0.05$ ).

## Discussion

The EMT is a key process in tissue development, carcinogenesis and organ fibrosis (41). In addition, it has emerged as a central mechanism underlying tubulointerstitial fibrosis, a progressive pathology common to numerous chronic kidney diseases, including DN (8,42). Interstitial myofibroblasts have a critical role in the development of tubulointerstitial fibrosis in diabetic and non-diabetic kidney diseases (43). A large proportion of interstitial myofibroblasts originate from transformed tubular epithelial cells experiencing pathological conditions during renal fibrogenesis (44). Interstitial fibrosis is characterized by *de novo* activation of  $\alpha$ -SMA and vimentin-positive myofibroblasts (45). Collagen I is a key component of the ECM. In the process of EMT, tubular epithelial cells acquire the myofibroblast marker  $\alpha$ -SMA and vimentin, display a myofibroblastic morphology and secrete interstitial matrix components such as collagen I and fibronectin (8). Previous studies have demonstrated that selective blockade of tubular EMT may protect the kidneys from developing fibrotic lesions following obstructive injury (46), and that tubular EMT has an important role in renal tubulointerstitial fibrosis (8,10,47).

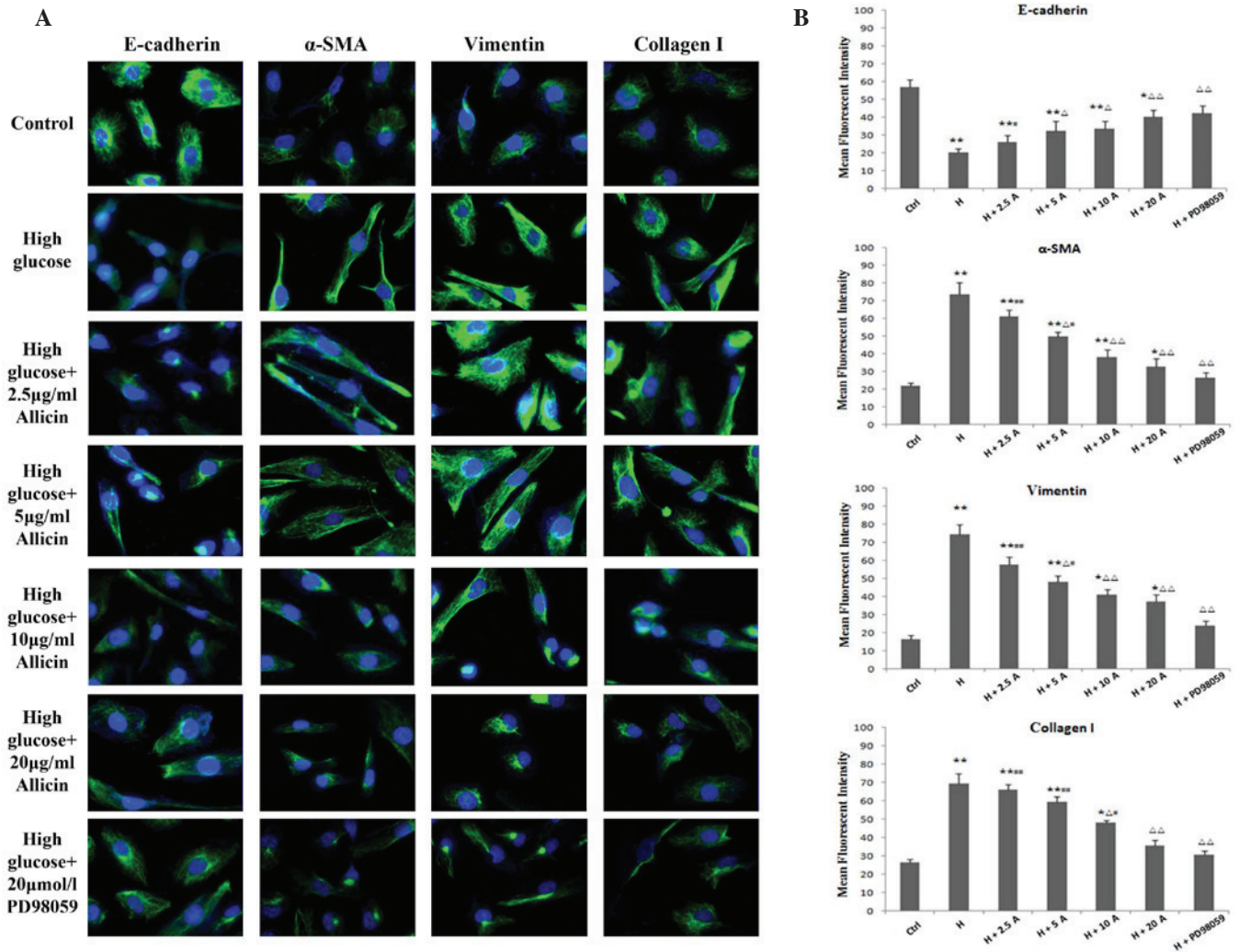


Figure 4. Expression of E-cadherin,  $\alpha$ -SMA, vimentin and collagen I in HK-2 cells. (A) Fluorescein isothiocyanate-labeled proteins are shown in green; DAPI-labeled nuclei are shown in blue. Cells were exposed to normal glucose (ctrl), high glucose, high glucose plus 5 or 2.5, 5, 10 and 20  $\mu$ g/ml allacin or high glucose plus 20  $\mu$ mol/l PD98059 for 48 h, as detected by fluorescence immunohistochemistry (magnification, x400). (B) Statistical analysis is also shown. \* $P$ <0.05 and \*\* $P$ <0.01 vs. Ctrl;  $^{\Delta}$  $P$ <0.05 and  $^{\Delta\Delta}$  $P$ <0.01 vs. H;  $^{\#}$  $P$ <0.05 and  $^{\#\#}$  $P$ <0.01 vs. H + PD98059.  $\alpha$ -SMA,  $\alpha$ -smooth muscle actin; Ctrl, control; H, high glucose; A, allacin.

Therefore, a potentially effective therapeutic strategy for progressive renal fibrosis may involve the prevention of tubular EMT in the diseased kidney.

TGF- $\beta$ 1 is a key mediator responsible for transdifferentiation *in vivo* and *in vitro* (7,46,48,49). It has previously been shown that TGF- $\beta$ 1 has an important role in altering the phenotype of renal epithelial cells, and that this significantly contributes to the profibrotic effects (50). Previous studies have demonstrated that advanced glycation end products, which accumulate in the diabetic kidney, are powerful mediators of EMT (51), and act via TGF- $\beta$ 1-dependent pathways involving various intracellular signaling molecules, including Smad and MAPK, in response to high glucose conditions (51,52). Our previous study reported overexpression of TGF- $\beta$ 1 during the EMT of renal tubular epithelial cells in a diabetic rat model (53). In the present study, it was shown that high glucose concentrations induced the EMT of HK-2 cells and significantly increased the expression levels of TGF- $\beta$ 1 and collagen I. These results suggested that the tubular EMT and increased ECM synthesis induced by hyperglycemia may at least partly depend on TGF- $\beta$ 1, while increased TGF- $\beta$ 1

secretion following transdifferentiation may form a positive feedback loop. Therefore, TGF- $\beta$ 1 may represent an additional key component of the pathway leading to EMT.

Activation of the Smad and/or MAPK signaling pathways is required for TGF- $\beta$ 1-induced EMT (54). In addition, phosphorylation of ERK1/2, a downstream signaling molecule of TGF- $\beta$ 1, is required for an optimal response to TGF- $\beta$ 1 (55). Rhyu *et al* (18) reported that PD98059, a specific inhibitor of the MAPK/ERK kinase signaling pathway, was able to effectively inhibit the TGF- $\beta$ 1-induced EMT process in NRK52E cells. In the present study, high glucose conditions activated the ERK1/2/MAPK and TGF- $\beta$  signaling pathways in the process of EMT. Previous studies have demonstrated that high glucose induces ERK1/2 phosphorylation *in vitro* and *in vivo* (56,57). Furthermore, high glucose-mediated activation of p-ERK1/2 and high-glucose induced EMT were shown to be blocked by PD98059 (58). These results suggested that blockade of high glucose-mediated activation of the ERK1/2/MAPK signaling pathway was able to inhibit the EMT, a critical process in renal tubulointerstitial fibrosis (59). Further studies are required in order to validate that high glucose mediates EMT via the

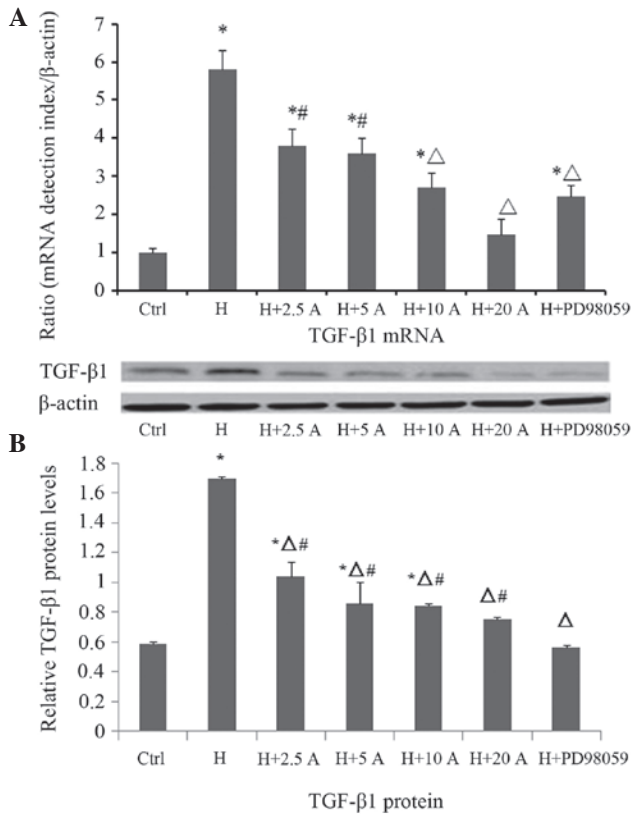


Figure 5. Allicin (2.5, 5, 10 or 20  $\mu$ g/ml) and PD98059 (20  $\mu$ mol/l) reversed the high glucose-induced upregulation of TGF- $\beta$ 1 mRNA and protein expression levels in HK-2 cells at 48 h. The (A) mRNA and (B) protein expression levels of TGF- $\beta$ 1 were determined by reverse transcription-quantitative polymerase chain reaction and western blotting, respectively. Data are presented as the mean  $\pm$  standard error of the mean. \*P<0.05 vs. the control;  $\Delta$ P<0.05 vs. the high glucose group; #P<0.05 vs. PD98059. TGF- $\beta$ 1, transforming growth factor- $\beta$ 1.

ERK/MAPK and TGF- $\beta$ 1 signaling pathways, which are involved in numerous intracellular processes.

Allicin, which is a major active component isolated from garlic, has been used as a popular folk medicine for thousands of years (60). Allicin has previously been shown to inhibit fibroblast proliferation and collagen synthesis by regulating the TGF- $\beta$  signaling pathway, and inhibited myocardial fibrosis caused by abdominal aortic coarctation via its inhibition of myocardial collagen (33,34). In a rat model of liver fibrosis, allicin was able to significantly inhibit the transdifferentiation of stellate cells to myofibroblasts via the downregulation of TGF- $\beta$ 1 expression (35). Zhang *et al* (36) demonstrated that allicin significantly attenuated the development of myocardial fibrosis and exerted significant anti-proliferative effects in rabbit arterial smooth muscle cells induced by angiotensin II in a dose- and time-dependent manner. These findings suggested that allicin may have a role in the prevention of tissue fibrosis. However, whether allicin has a role in preventing renal fibrosis remains unknown.

The present study demonstrated that allicin was able to block the EMT and decrease the expression levels of collagen I in HK-2 cells cultured under high glucose conditions. Notably, 25 mM glucose was able to induce the transdifferentiation of tubular cells into myofibroblasts that showed fibroblast-like morphologies, a loss of E-cadherin epithelial marker expression

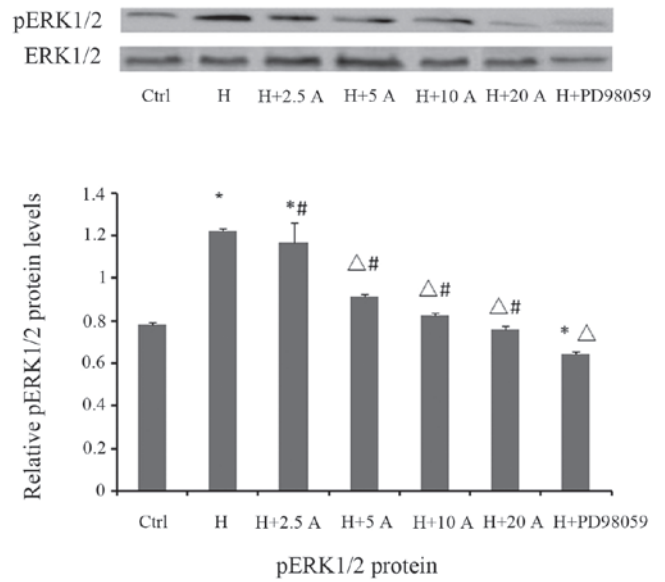


Figure 6. Allicin (2.5, 5, 10 or 20  $\mu$ g/ml) and PD98059 (20  $\mu$ mol/l) reversed the high glucose-induced upregulation of p-ERK1/2 protein expression in HK-2 cells at 48 h, as shown by western blotting and densitometry. Data are presented as the mean  $\pm$  standard error of the mean. \*P<0.05 vs. the control;  $\Delta$ P<0.05 vs. the high glucose group; #P<0.05 vs. PD98059. p-ERK1/2, phosphorylated-extracellular signal regulated kinase 1/2.

and  $\alpha$ -SMA and vimentin positivity. In addition, collagen I expression was shown to be increased in high glucose-induced HK-2 cells, which indicated that the transformed cells had begun to produce components of the ECM. Importantly, allicin treatment increased the expression of E-cadherin, prevented the *de novo* expression of  $\alpha$ -SMA and vimentin, and reduced collagen I expression in a dose-dependent manner. Furthermore, the present study demonstrated that simultaneous incubation of HK-2 cells with allicin markedly decreased the expression of p-ERK1/2 at 48 h in a dose-dependent manner, in particular at 20  $\mu$ g/ml. In addition, allicin reduced the expression of TGF- $\beta$ 1, potentially by inhibiting the high glucose-mediated activation of the ERK1/2 signaling pathway, thereby inhibiting HK-2 cell morphological changes, the EMT and ECM synthesis, and resulting in the attenuation of tubular fibrosis.

In conclusion, the present study demonstrated that high glucose concentrations induced the EMT of renal tubule epithelial cells, and this was associated with upregulation of TGF- $\beta$ 1 and collagen I. In addition, it was shown that the MAPK inhibitor PD98059 was able to reverse high glucose-induced transdifferentiation of HK-2 cells by inhibiting the expression of p-ERK1/2 and TGF- $\beta$ 1. These results suggested that TGF- $\beta$ 1 is an important regulator of the EMT and that ERK1/2 signaling pathway may be involved in renal interstitial fibrosis associated with DN. Furthermore, allicin treatment restrained the EMT and prevented subsequent interstitial matrix accumulation *in vitro*. However, further studies are required in order to clarify the effects of allicin on renal fibrosis.

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