Combined treatment with vitamin E and gefitinib has synergistic effects to inhibit TGF-β1-induced renal fibroblast proliferation

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Abstract. Renal fibroblast proliferation is key in renal fibrosis and chronic kidney disease. Transforming growth factor-β1 (TGF-β1) has been demonstrated to be an important factor that induces cell proliferation in renal fibroblasts. Epidermal growth factor receptor (EGFR) is also recognized as a factor promoting renal fibroblast proliferation. In addition, mitogen-activated protein kinase signaling pathways are associated with TGF-β1- and EGFR-induced cell proliferation. Gefitinib, an EGFR tyrosine kinase inhibitor, is predominantly used as an anti-tumor therapeutic agent in clinical therapeutic strategies. However, gefitinib has been suggested to exert anti-proliferative effects on renal fibroblasts, however, high-dose gefitinib may result in serious side effects. The present study aims to determine whether low-dose gefitinib reduces gefitinib-induced side effects and maintains the anti-proliferative effects on renal fibroblasts. TGF-β1 promotes cell proliferation in renal fibroblasts, and the current study demonstrates that low-dose gefitinib treatment exhibits anti-proliferative effects similar to those of high-dose gefitinib treatment. Thus, although high-dose gefitinib is a conventional anti-tumor drug, low-dose gefitinib may be of use in renal fibrosis treatment. Furthermore, the present study demonstrates that a combined treatment with low-dose gefitinib and vitamin E has synergistic effects that reduce TGF-β1-induced fibroblast proliferation, cell-cycle arrest and the ERK phosphorylation pathway.

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Introduction

Renal fibroblast proliferation induces tubulointerstitial fibrosis resulting in renal filtration dysfunction (1) and chronic kidney disease (CKD) (2,3), thus, the inhibition of fibroblast proliferation to prevent CKD is an important area. Transforming growth factor-β1 (TGF-β1) is important in the induction of proliferation in human renal fibroblasts (4,5). Previous studies have suggested that induction of renal fibrosis by TGF-β1 is associated with p53 (6,7), reactive oxygen species (8,9), the Smad signaling pathway (10,11), mitogen activated protein kinase (MAPK) signaling pathways (12,13), and RhoA/Rho kinase (9,14). These studies indicated that TGF-β1 is a critical factor in activating numerous signal transduction pathways that result in proliferation in renal fibroblasts. Thus, TGF-β1 was used in the present study as a cell model for investigating anti-proliferative effects on renal fibroblasts by gefitinib and vitamin E treatment alone and in combination. Results from the current study demonstrated that 0.2 nM TGF-β1 promoted renal fibroblast proliferation.

The epidermal growth factor receptor (EGFR) signaling pathway induces cell proliferation in various cells (15-18). Previous studies have demonstrated that the EGFR signaling pathway mediates renal fibroblast proliferation and renal fibrogenesis (19,20). Gefitinib, an EGFR tyrosine kinase inhibitor, inhibits EGFR signaling activation resulting in cell growth arrest (21,22). Thus, gefitinib has generally been used for clinical tumor treatment (23-25). As EGFR mediates renal fibroblast proliferation and EGFR is blocked by gefitinib, a previous study has successfully used gefitinib to inhibit renal fibroblast proliferation (26). The present study demonstrated that gefitinib attenuates fibroblast proliferation by blocking the EGFR signaling pathway and by inhibiting the TGF-β1-mediated pathway. In addition, previous studies have suggested that the EGFR signaling pathway is associated with the TGF-β1-mediated pathway (8,27). Similar to these studies, experimental data from the present study also demonstrated that gefitinib inhibits TGF-β1-induced fibroblast proliferation. Although gefitinib effectively inhibits fibroblast proliferation to prevent renal fibrosis, the

side effects as a result of gefitinib are also clinically important (28-31).

Vitamin E exerts an anti-oxidative and protective effect against various oxidative stress-associated diseases, including hypertension, cardiovascular disease, hemorrhagic liposis, and obesity-associated diseases (32-35). However, vitamin E exerts an anti-fibrotic effect on the renal cell-mediated TGF-β1 signaling pathway (36-38). A previous study indicated that vitamin E reduces progression of fibrosis in obstructed kidneys (36). Other previous studies have demonstrated that vitamin E in combination with pentoxifylline or Fuzheng Huayu recipe, a traditional Chinese medicine, inhibits TGF-β1-induced fibrosis (37,38). Results from the present study also demonstrated that vitamin E inhibits cell proliferation in TGF-β1-treated renal fibroblasts. Furthermore, the present study indicates that a combination treatment of low-dose vitamin E and low-dose gefitinib has a more marked anti-proliferative effect on TGF-β1-treated renal fibroblasts than high-dose vitamin E treatment or high-dose gefitinib treatment alone. This suggests that combination treatment with low-dose vitamin E and low-dose gefitinib is a potential therapeutic strategy to inhibit fibroblast proliferation and prevent high-dose gefitinib treatment-induced side effects.

Three major MAPK signaling pathways contain extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase, and p38 mitogen-activated protein kinase (39,40). Previous studies have suggested that renal fibroblast proliferation is mediated by MAPK signaling pathways (41,42). The present study demonstrates that ERK phosphorylation is increased in TGF-β1-treated renal fibroblasts, suggesting TGF-β1-induced proliferation is mediated by the ERK signaling pathway. In addition, the present study demonstrated that combination treatment of low-dose vitamin E and low-dose gefitinib reduces TGF-β1-induced increases in ERK phosphorylation levels. The present study indicates that combination treatment with low-dose gefitinib and low-dose vitamin E has synergistic effects to inhibit TGF-β1-induced renal fibroblast proliferation mediated by the ERK phosphorylation signaling pathway.

Materials and methods

Materials. TGF-β1 was obtained from R&D Systems, Inc. (Minneapolis, MN, USA). Anti-ERK (1:400; cat. no. BS3627), anti-p-ERK (1:400; cat. no. BS5016), anti-p38 (1:400; cat. no. BS3567) and anti-p-p38 (1:400; cat. no. BS4766) primary rabbit polyclonal antibodies were purchased from Bioworld (Louis Park, MN, USA). Horseradish peroxidae-conjugated goat anti-rabbit IgG, secondary antibody (1:2,000, cat. no. 7074) was purchased from Cell Signaling Technology (Danvers, MA, USA). 5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay kit was bought from Bio Basic Canada, Inc. (Markham, ON, Canada). Fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), non-essential amino acids, L-glutamine, and penicillin/streptomycin were purchased from Hyclone (GE Healthcare Life Sciences, Logan, UT, USA). Vitamin E and dimethyl sulfoxide (DMSO) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Gefitinib was purchased from AstraZeneca UK Limited (London, UK).

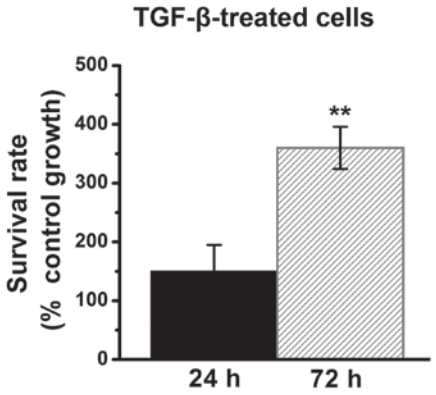

Figure 1. Cell survival rate. NRK-49F cells were treated with 0.2 nM TGF-β1. The survival rate is ~150% after 24 h of TGF-β1 treatment, and ~360% after 72 h of TGF-β1 treatment. The data was analyzed from four independent experiments and presented as the mean ± standard deviation. **P<0.01 vs. the 24 h group. TGF-β1, transforming growth factor-β1.

Cell line and cell culture. The NRK-49F rat renal fibroblast cell line was obtained from Bioresource Collection and Research Center (Hsinchu, Taiwan). The cell line was cultured in DMEM supplemented with 10% FBS, 2 mM L-glutamine, 100 IU/ml penicillin/streptomycin, and 0.1 mM non-essential amino acids, and maintained in a humidified atmosphere with 5% CO_2 at 37˚C.

Cell survival rate assay. Survival rates of NRK-49F cells were measured with the MTT assay method as described in previous studies (43,44). Briefly, cells were cultured in 96-well plates. On the second day, cells were divided into control group and experimental groups and MTT assays (with DMSO treatment). were determined at 24 and 72 h according to the manufacturer's protocols. Absorbance was determined under a multi-well ELISA reader (SpectraMax Paradigm Multi-Mode Microplate Reader; Molecular Devices, Sunnyvale, CA, USA) at a wavelength of 570 nm. Survival rates were indicated using the following formula: A570 experimental group / A570 control group.

Cell cycle analysis. Cell cycle analysis was conducted using fluorescence-activated cell sorting as described previously (45,46). Briefly, NRK-49F cells from the control and experimental groups were collected and washed with phosphate-buffered saline (PBS; containing 140 mM NaCl, 2.5 mM KCl, 15 mM Na_2HPO_4 and 1.6 mM KH_2PO_4), then fixed with 70% ethanol (Echo Chemical Co., Ltd., Miaoli, Taiwan) at 4˚C for 1 h. The fixed cells were washed with PBS and then treated with 1 ml propidium iodide (PI) solution (50 μg/ml PI, 100 μg/ml RNase A, and 0.1% Triton X-100) for 30 min at 37˚C. Following this, cells were washed with PBS and analyzed by flow cytometry (Partec CyFlow® SL; Sysmex Partec GmbH, Görlitz, Germany). The resulting data was analyzed with WinMDI version 2.8 software (http://winmdi.software.informer.com/2.8/).

Sodium dodecyl sulfate (SDS) electrophoresis and western blotting. Gel electrophoresis and western blotting were performed as previously described (47,48). Briefly, cells were treated with lysis buffer (containing 50 mM Tris-HCl,

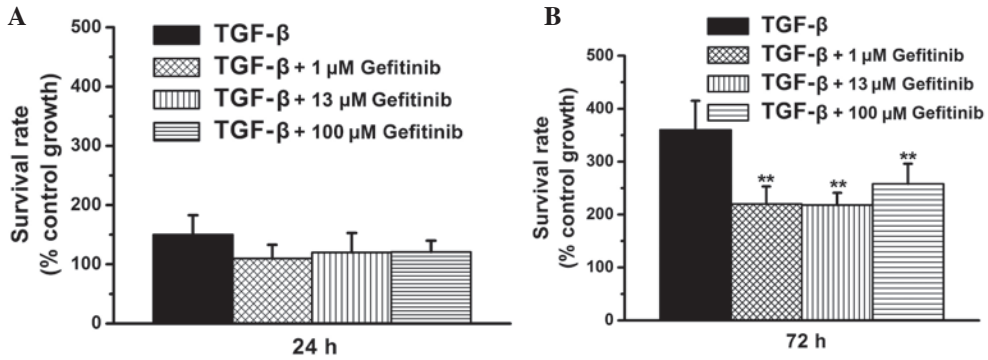

Figure 2. Cell survival rates. (A) 24 h and (B) 72 h survival rates of NRK49-F cells were calculated in the 0.2 nM TGF-β1-treated, 0.2 nM TGF-β1 with 1 μM gefitinib-treated, 0.2 nM TGF-β1 with 13 μM gefitinib-treated and 0.2 nM TGF-β1 with 100 μM gefitinib-treated groups. At 72 h, the survival rate is markedly lower in TGF-β1 with gefitinib-treated groups than in TGF-β1 without gefitinib-treated group. The data was analyzed from four independent experiments and presented as the mean ± standard deviation. **P<0.01 vs. the TGF-β1 group. TGF-β1, transforming growth factor-β1.

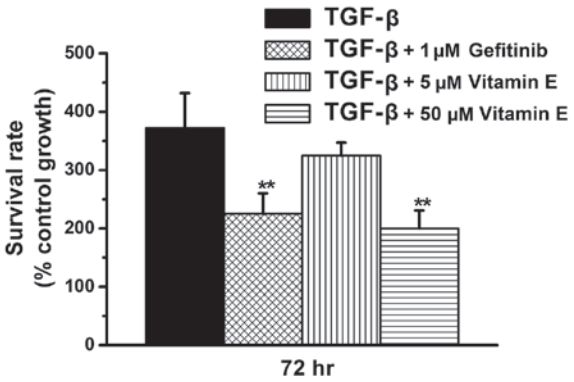

Figure 3. Cell survival rates. The 72 h survival rates of NRK49-F cells were calculated in the 0.2 nM TGF-β1-treated, 0.2 nM TGF-β1 with 1 μM gefitinib-treated, 0.2 nM TGF-β1 with 5 μM vitamin E-treated and 0.2 nM TGF-β1 with 50 μM vitamin E-treated groups. The survival rate is markedly lower in the TGF-β1 with gefitinib-treated group and the TGF-β1 with 50 μM vitamin E-treated group compared with the TGF-β1-treated group. The data was analyzed from four independent experiments and presented as the mean ± standard deviation. **P<0.01 vs. the TGF-β1 group. TGF-β1, transforming growth factor-β1.

120 mM NaCl, 1 mM EDTA and 1% NP-40) and centrifuged at 16,000 x g for 10 min at 4˚C. The supernatant layer containing proteins was collected and the protein level was determined using a Bicinchoninic Acid Protein Assay Reagent kit (Pierce Biotechnology, Rockford, IL, USA) with a DU 530 spectrophotometer (OD 562 nm; Beckman Coulter, Inc., Brea, CA, USA). Equal quantities of protein (60 μg) were loaded and run on an SDS-PAGE for 45 min and transferred to a PVDF membrane. The membranes were blocked with 5% milk for 2 h and washed three times with PBS. The membranes were incubated with primary antibodies in 5% milk for 2 h. The membranes were then washed with PBS three times and incubated with secondary antibodies for 1 h. Protein levels were analyzed with Western Lightning® Chemiluminescence Plus reagent (PerkinElmer, Inc., Waltham, MA, USA) and were observed with a Luminescence Image Analysis system (LAS-4000, FUJIFILM Electronic Materials Taiwan Co., Ltd., Tainan, Taiwan).

Statistical analysis. Data were measured from four independent experiments and are presented as the mean ± standard deviation. The data was analyzed using a Student's t-test with Excel 2010 (http://microsoft-excel-2010.updatestar.com/zh-tw). P<0.05 was considered to indicate a statistically significant difference between two groups.

Results

TGF-β1 induces renal fibroblast proliferation in a time-dependent manner. Consistent with data from previous studies (4,5), the data from the present study demonstrates that TGF-β1 induces proliferation in renal fibroblasts. Compared with growth in the control cells (without TGF-β1 treatment), the survival rate is ~150% in TGF-β1-treated cells at 24 h. However, the survival rate is significantly increased by >360% in TGF-β1-treated cells at 72 h (P<0.01; Fig. 1). The present study suggested that TGF-β1 induces cell proliferation in renal fibroblasts in a time-dependent manner. The present study then used TGF-β1-induced cell proliferation as an experimental model to investigate the antiproliferative effects of gefitinib treatment, vitamin E treatment, and combination treatment of gefitinib and vitamin E on renal fibroblasts.

Gefitinib exerts antiproliferative effects on TGF-β1-treated renal fibroblasts. The present study aimed to investigate whether gefitinib inhibits TGF-β1-induced cell proliferation. The anti-proliferative effects of gefitinib (high dose, 100 μM; low dose for clinical tumor treatment, 13 μM; and low dose, 1 μM) were examined in TGF-β1-treated renal fibroblasts. Compared with the control group, the 24-h survival rates in the present study were ~150% in the TGF-β1-treated group and ~100% in the TGF-β1 + gefitinib-treated groups (Fig. 2A). In addition, the 72-h survival rates were >360% in the TGF-β1-treated group and <250% in the TGF-β1 + gefitinib-treated group (P<0.01; Fig. 2B). Results from the present study demonstrated that gefitinib reduces TGF-β1-induced cell proliferation. Furthermore, as shown in Fig. 2, there is no marked difference in survival rates among the TGF-β1 + gefitinib (100, 13 and 1 μM)-treated groups at 24 and 72 h. The data from the present study suggested that high- and low-dose gefitinib (100, 13 and 1 μM) are equally effective at inhibiting TGF-β1-induced cell proliferation (as shown in Fig. 2B).

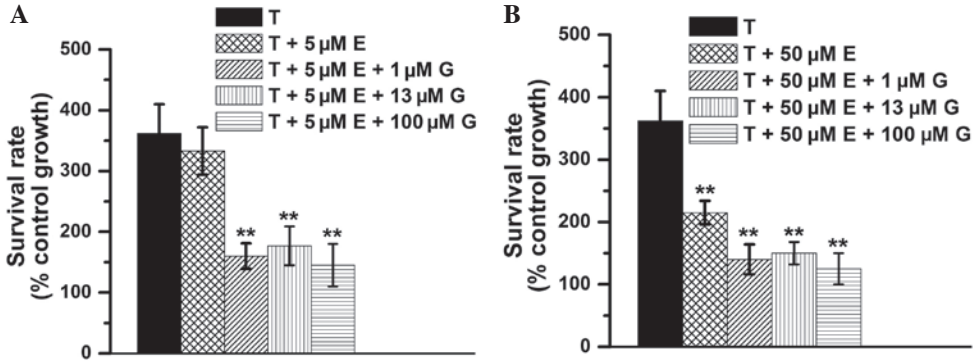

Figure 4. Cell survival rates. (A) The 72 h survival rates of NRK49-F cells were calculated in the 0.2 nM TGF-β1-treated, 0.2 nM TGF-β1 with 5 μM vitamin E-treated, 0.2 nM TGF-β1 with 5 μM vitamin E + 1 μM gefitinib-treated, 0.2 nM TGF-β1 with 5 μM vitamin E + 13 μM gefitinib-treated and 0.2 nM TGF-β1 with 5 μM vitamin E + 100 μM gefitinib-treated groups. (B) The 72 h survival rates of NRK49-F cells were calculated in the 0.2 nM TGF-β1-treated, 0.2 nM TGF-β1 with 50 μM vitamin E-treated, 0.2 nM TGF-β1 with 50 μM vitamin E + 1 μM gefitinib-treated, 0.2 nM TGF-β1 with 50 μM vitamin E + 13 μM gefitinib-treated, and 0.2 nM TGF-β1 with 50 μM vitamin E + 100 μM gefitinib-treated group. The data was analyzed from four independent experiments and presented as the mean ± standard deviation. **P<0.01 vs. the TGF-β1 group. TGF-β1 (T), transforming growth factor-β1; E, vitamin E; G, gefitinib.

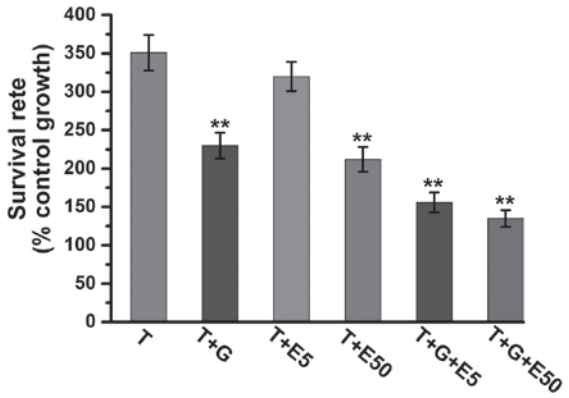

Figure 5. Cell survival rates. The 72 h survival rates of NRK49-F cells were calculated in the 0.2 nM TGF-β1-treated, 0.2 nM TGF-β1 with 1 μM gefitinib-treated, 0.2 nM TGF-β1 with 5 μM vitamin E-treated, 0.2 nM TGF-β1 with 50 μM vitamin E-treated, 0.2 nM TGF-β1 with 1 μM gefitinib + 5 μM vitamin E-treated, and 0.2 nM TGF-β1 with 1 μM gefitinib + 50 μM vitamin E-treated groups. The data was analyzed from four independent experiments and presented as the mean ± standard deviation. **P<0.01 vs. the TGF-β1 group. TGF-β1 (T), transforming growth factor-β1; E, vitamin E; G, gefitinib.

Vitamin E reduces cell proliferation in TGF-β1-treated renal fibroblasts in a dose-dependent manner. A previous study indicated that vitamin inhibits the progression of fibrosis in obstructed kidneys (36). Numerous other studies have demonstrated that epithelial-mesenchymal transition (EMT) and fibroblast proliferation induce renal fibrosis (49-52). The present study further determined whether vitamin E inhibits fibroblast proliferation directly to prevent progression of fibrosis. As presented in Fig. 3, the 72-h survival rate is >360% in the TGF-β1-treated group, ~330% in the TGF-β1 + 5 μM vitamin E-treated group, and <230% in the TGF-β1 + gefitinib-treated group and the TGF-β1 + 50 μM vitamin E-treated group. The present study demonstrated that high-dose vitamin E (50 μM) treatment reduces TGF-β1-induced cell proliferation (P<0.01 vs. the TGF β1 group, Fig. 3) similarly to gefitinib treatment. However, low-dose vitamin E (5 μM) treatment did not markedly reduce TGF-β1-induced cell proliferation. Thus, the results from the present study suggest that vitamin E reduces TGF-β1-induced renal cell proliferation in a dose-dependent manner.

Gefitinib enhances the antiproliferative effects of vitamin E on TGF-β1-treated renal fibroblasts. The present study aimed to investigate whether gefitinib promotes the antiproliferative effects of vitamin E on TGF-β1-treated cells. The 72-h survival rate was >360% in the TGF-β1-treated group, ~330% in the TGF-β1 + 5 μM vitamin E-treated group, and ~160% in TGF-β1 + 5 μM vitamin E with various concentrations of gefitinib-treated groups (Fig. 4A). In addition, the 72-h survival rate was >360% in the TGF-β1-treated group, ~220% in the TGF-β1 + 50 μM vitamin E-treated group, and ~150% in the TGF-β1 + 50 μM vitamin E with various concentrations of gefitinib-treated groups (Fig. 4B). These data suggest that gefitinib enhances the antiproliferative effects of vitamin E on TGF-β1-treated cells. However, the antiproliferative effects were not markedly different among those treated with vitamin E + various concentrations (1, 13 and 100 μM) of gefitinib. Furthermore, the data demonstrated that although low-dose vitamin E does not have notable antiproliferative effects, combination treatment of low-dose vitamin E and gefitinib effectively reduces TGF-β1-induced cell proliferation (P<0.01 vs. the TGF-β1 group, Fig. 4A).

Combination treatment of low-dose gefitinib and low-dose vitamin E has synergistic effects to reduce TGF-β1-induced renal fibroblast proliferation. As presented in Figs. 2 and 3, gefitinib and vitamin E have been demonstrated to exert anti-proliferative effects on TGF-β1-treated cells. The current study further analyzed the anti-proliferative effects on TGF-β1-induced cell proliferation in the gefitinib-treated group, the vitamin E-treated group, and the gefitinib + vitamin E-treated group. As presented in Fig. 5, the 72-h survival rate was >360% in TGF-β1-treated cells, ~340% in TGF-β1 with low-dose vitamin E-treated group, and ~250% in TGF-β1 with high-dose vitamin E-treated or gefitinib groups. These data indicate that low-dose vitamin E does not have marked anti-proliferative effects on TGF-β1-induced cell proliferation; however, high-dose vitamin E and low-dose gefitinib have similar anti-proliferative effects on

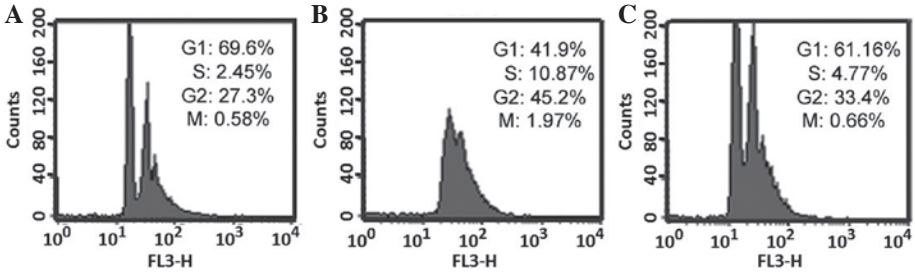

Figure 6. Cell cycle analysis. The cell cycle was analyzed at 24 h by flow cytometry in (A) Control cells, (B) 0.2 nM TGF-β1-treated cells and (C) 0.2 nM TGF-β1 with 1 μM gefitinib + 5 μM vitamin E-treated cells. The S-phase percentage was markedly increased in TGF-β1-treated cells, however, treatment with a combination of gefitinib and vitamin E decreased TGF-β1-induced S-phase percentage increases. TGF-β1 (T), transforming growth factor-β1.

TGF-β1-induced cell proliferation. Furthermore, the present study demonstrated that the 72-h survival rates are ~160% in TGF-β1-induced cells with low-dose gefitinib + high-dose or low-dose vitamin E-treated groups. These data indicate that the anti-proliferative effects in combination treatment with low-dose gefitinib and low-dose vitamin E is similar to combination treatment with low-dose gefitinib and high-dose vitamin E. Furthermore, the combination treatment with gefitinib and vitamin E has stronger anti-proliferative effects than gefitinib treatment alone or vitamin E treatment alone. Thus, the results of the present study suggest that combination treatment of low-dose gefitinib and low-dose vitamin E reduces TGF-β1-induced cell proliferation ($P<0.01$ vs. the TGF-β1 group, Fig. 5).

Combination treatment with gefitinib and vitamin E reduces TGF-β1-induced cell proliferation associated with the cell cycle and ERK signaling pathway. The cell cycle was analyzed in the control group, the TGF-β1-treated group, and the TGF-β1 with gefitinib + vitamin E-treated group. As presented in Fig. 6A, the G_1 phase was ~69.6% and the S-phase is ~2.45% in the control group. As presented in Fig. 6B, the G_1 phase was ~41.9% and the S-phase was ~10.87% in TGF-β1-treated group. As presented in Fig. 6C, the G_1 phase was ~61.16% and the S-phase was ~4.77% in TGF-β1 with gefitinib + vitamin E-treated group. All data obtained from flow cytometry were analyzed using Student's t-test. The S-phase percentage was significantly increased in the TGF-β1-treated group compared with the control ($P<0.05$, as determined from four independent flow cytometry experiments, data not shown), this indicates that TGF-β1 accelerate entry to S-phase, resulting in cell proliferation. Furthermore, S-phase percentage is significantly increased in the TGF-β1-treated group compared with the TGF-β1 with gefitinib + vitamin E-treated group. The result suggested that combination treatment with gefitinib and vitamin E may ameliorate the increase in cells entering the S-phase in TGF-β1-treated cells. Furthermore, previous studies have demonstrated MAPK signaling pathways, including ERK and p38 phosphorylation, are associated with renal fibroblast proliferation (41,42). Thus, ERK and p38 phosphorylation, p-ERK and p-p38, were analyzed in the control group, the TGF-β1-treated group, and the TGF-β1 with gefitinib + vitamin E-treated group (Fig. 7). Results from the present study demonstrated that p-ERK was not observed in the control group (Fig. 7, lane 1), but is evident in the TGF-β1-treated group (Fig. 7, lane 2). This suggests

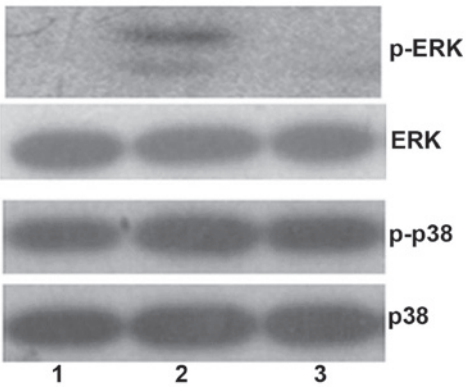

Figure 7. Western blot analysis. ERK and p38 phosphorylation were analyzed at 2 h. p-ERK and p-p38 indicated the phosphorylation levels of ERK and p38, respectively. ERK and p38 were internal controls. p-ERK was observed in TGF-β1-treated cells, however, p-ERK was not observed in the control or TGF-β1 with gefitinib and vitamin E treated groups. Lane 1, control cells; lane 2, 0.2 nM TGF-β1-treated cells; lane 3, 0.2 nM TGF-β1 with 1 μM gefitinib + 5 μM vitamin E-treated cells.

TGF-β1 may induce renal cell proliferation may be via ERK phosphorylation. However, p-ERK was also not observed in the TGF-β1 with gefitinib + vitamin E-treated group (Fig. 7, lane 3). The data indicated gefitinib + vitamin E treatment inhibits ERK phosphorylation. However, the p-p38 levels were not significantly different among the three groups. The results from the current study suggest that combination treatment with gefitinib + vitamin E reduces TGF-β1-induced cell proliferation associated with ERK phosphorylation.

Discussion

Gefitinib, an EGFR tyrosine kinase inhibitor, inhibits cell growth (21,22) and has been used for various tumor treatments, including, lung, esophageal and breast cancer (25,29,53). Numerous studies have demonstrated that therapeutic doses of gefitinib for clinical tumor treatment result in side effects, including severe hepatotoxicity (29), acneiform eruption, severe xerosis of skin, paronychia (30), and empyema (31). However, in the present study, the results indicated that there are similar anti-proliferative effects on TGF-β1-treated renal fibroblasts among high-dose, therapeutic dose, and low-dose gefitinib treatments (Fig. 2). The results of the present study indicate that gefitinib, a conventional therapeutic agent for tumor treatment, may be useful for the treatment of renal fibrosis at a low-dose.

Multiple studies have demonstrated that renal fibrosis is induced via the EMT process and renal fibroblast proliferation (49-52). In addition, it has been reported that EMT and fibroblast proliferation are induced by activation of the TGF-β1 signaling pathway (54-57). Similar to these studies, data from the present study also showed that TGF-β1 induces renal fibroblast proliferation. Previous research has indicated that vitamin E in combination with other therapeutic agents reduces progression of TGF-β1-induced fibrosis (37,38). The current study further demonstrated that vitamin E alone inhibits TGF-β1-induced fibroblast proliferation (Fig. 3). In addition, high-dose vitamin E, like gefitinib, has a more marked anti-proliferative effect than low-dose vitamin E. Although the anti-fibrotic effects exerted by vitamin E remain to be elucidated, the present study demonstrated that vitamin E reduces proliferation in TGF-β1-treated fibroblasts.

Results from the present study demonstrate that combination treatment with gefitinib and vitamin E has an increased anti-proliferative effect on TGF-β1-treated cells compared with gefitinib or vitamin E treatment alone. Furthermore, these results also demonstrated that combination treatment with low-dose gefitinib and low-dose vitamin E has anti-proliferative effects similar to combination treatment with high-dose gefitinib and high-dose vitamin E. The results of the current study demonstrate that low-dose gefitinib and low-dose vitamin E treatment may be a potential therapeutic strategy for renal fibrosis and an effective alternative to avoid high-dose gefitinib-induced side effects.

Previous studies have suggested that TGF-β1 induces cell cycle-associated protein expression (4,58) and activates MAPK signaling pathways (59,60). Similar to these studies, the results from the present study have also demonstrated that TGF-β1 promotes cells to enter the S-phase and activate ERK phosphorylation. In addition, the present study also demonstrated that combination treatment with low-dose gefitinib and vitamin E induces G_1 arrest and reduces ERK phosphorylation levels to inhibit TGF-β1-induced proliferation.

In conclusion, the present study demonstrated that combination treatment with low-dose gefitinib and vitamin E has anti-proliferative effects on TGF-β1-treated fibroblasts via cell cycle arrest and inactivation of the ERK signaling pathway.

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