Activation of GPR40 attenuates apoptosis and EMT induced by TNF-α in rat proximal tubular cells

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OBJECTIVES

- Apoptosis and epithelial-mesenchymal transition (EMT) of renal tubular epithelial cells are the final common pathogenesis of chronic progressive kidney disease. Tumor necrosis factor (TNF)-α plays a crucial role in these pathologic changes of renal tubular epithelial cells.
- G-protein-coupled receptor 40 (GPR40) is a member of a subfamily of G-protein-coupled receptors. Recently, diverse pharmacological effects of GPR40 agonists have been observed including anti-apoptosis and inhibition of TNF-α.
- However, no previous studies have investigated expression and function of GPR40 in the mammalian kidney.
- The present study investigated the changes in the expression of GPR40 in the obstructed kidney of mice with unilateral ureteral obstruction (UUO). Furthermore, we also investigated the effects of GPR40 activation on the apoptosis and EMT induced by TNF-α in rat proximal tubular (NRK52E) cells.

METHODS

- UUO was induced in C57BL/6J mice for 2 weeks. The protein expressions of GPR40, Bax, Bcl-2, transforming growth factor (TGF)-β1 and α-smooth muscle actin (SMA) were determined in the kidney.
- NRK52E cells were cultured with TNF-α (20 ng/mL) in the absence or presence of GW9508 (10 μM), a selective GPR40 agonist. Cell viability was evaluated by the MTT assay. We determined the protein expression of Bax, Bcl-2, TGF-β1, α-SMA and connective tissue growth factor (CTGF) by immunoblotting. We also determined the protein expression of Src, EGFR and ERK.

RESULTS

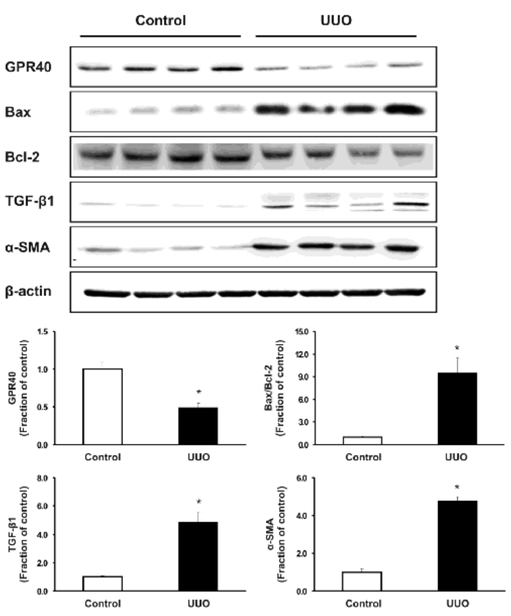

Figure 1. The protein expression of GPR40 was decreased in the ureteral obstructed kidney of mice with UUO, but the expression levels of Bax/Bcl-2, TGF-β1 and α-SMA were increased. *p< 0.05 vs. control.

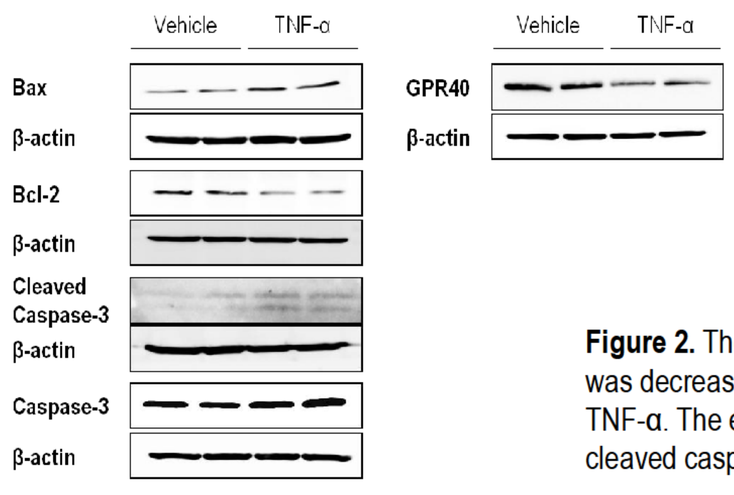

Figure 2. The protein expression of GPR40 was decreased in NRK52E cells treated with TNF-α. The expressions of Bax/Bcl-2 and cleaved caspase-3 were increased.

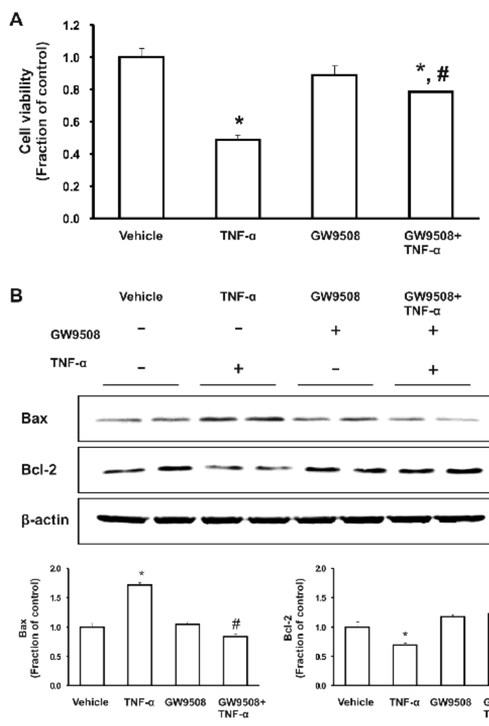

Figure 3. (A) MTT assay showed that the pretreatment of GW9508 attenuated the decreased cell viability by TNF-α in NRK52E cells. (B) TNF-α treatment increased the expression of Bax but decreased Bcl-2 expression, which was restored by the pretreatment with GW9508. *p< 0.05 vs. vehicle treated cells, #p<0.05 vs. TNF-α treated cells.

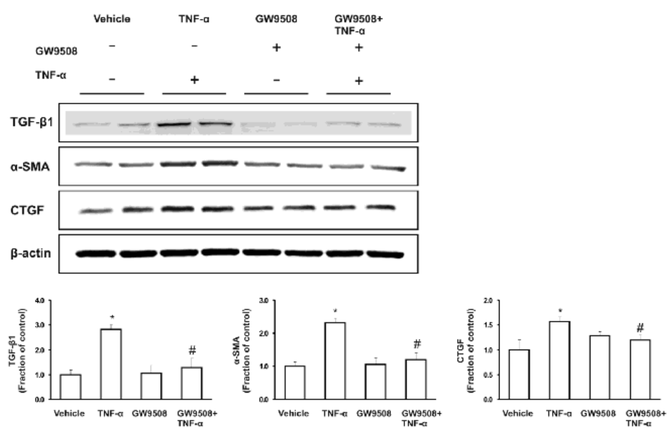

Figure 4. The protein expression of TGF-β1, α-SMA and CTGF was increased after the treatment of TNF-α in NRK52E cells, which was prevented by GW9508 pretreatment. *p< 0.05 vs. vehicle treated cells, #p<0.05 vs. TNF-α treated cells.

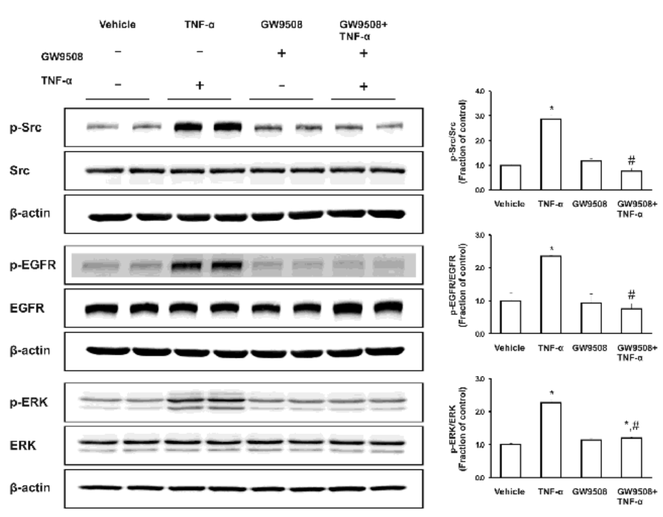

Figure 5. TNF-α treatment increased the phosphorylation of Src/EGFR/ERK in NRK52E cells, which was counteracted by GW9508 pretreatment. *p< 0.05 vs. vehicle treated cells, #p<0.05 vs. TNF-α treated cells.

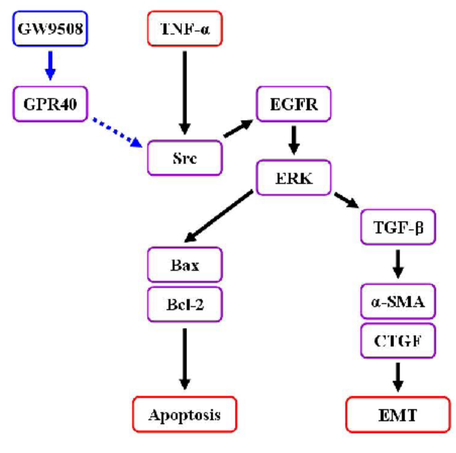

Figure 6. Proposed mechanism for the role of GPR40 activation in apoptosis and EMT induced by TNF-α in NRK52E cells.

CONCLUSIONS

- The protein expression of GPR40 was decreased in the obstructed kidney of mice with UUO.
- In NRK52E cells, GPR40 activation attenuates cell death induced by TNF-α through the inhibition of Src/EGFR/ERK, pro-apoptotic proteins, TGF-β1, α-SMA and CTGF.

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