Supplementary Materials

Histologic and morphologic analysis

Histologic and morphologic analysis was performed as previously described [1]. Immunohistochemical staining was performed using primary antibodies against phosphorylated-Smad3 (p-Smad3) (1:500; Santa Cruz Biotechnology, Santa Cruz, CA, USA), fibronectin (1:500; BD Biosciences, San Jose, CA, USA), α-smooth muscle actin (α-SMA) (1:500; Sigma, St. Louis, MO, USA), plasminogen activator inhibitor 1 (PAI-1) (1:500; BD Biosciences), and type I collagen (1:500; Abcam, Cambridge, UK), and horseradish peroxidase-conjugated anti-mouse or anti-rabbit immunoglobulin G secondary antibodies (Dako, Glostrup, Denmark). Quantification of renal fibrosis was performed as previously described [1].

Cell culture

Normal rat kidney fibroblasts (NRK-49F) and renal proximal tubular epithelial cells of human and rat (human proximal renal tubular epithelial [HK-2] and NRK-52E) origin were purchased from the American Type Culture Collection (Manassas, VA, USA). NRK-49F and NRK-52E cells were cultured as described previously [1,2]. HK-2 cells were cultured in 5% CO_2/95% air at 37°C in Keratinocyte Serum Free Medium (GIBCO™; Thermo Fisher, Vilnius, Lithuania). The medium was supplemented with both of the two additives required to grow this cell line: bovine pituitary extract and human recombinant epidermal growth factor. Cells were rendered quiescent by incubation for 24 hours in medium supplemented with 0.5% fetal bovine serum (FBS) and were then incubated in medium containing 0.5% FBS±transforming growth factor-β (5 ng/mL; R&D Systems, Minneapolis, MN, USA) ±evogliptin (100 or 200 μg/mL) for 24 hours. Cell lysates were then prepared, as described below.

Western blot analysis and *in vitro* transient transfection and reporter assay

Western blotting was performed as previously described [1]. Membranes were incubated with anti-p-Smad3 (1:1,000; Cell Signaling Technology, Danvers, MA, USA), anti-Smad3 (1:1,000; Cell Signaling Technology), anti-PAI-1 (1:1,000; BD Biosciences), anti-fibronectin (1:1,000; BD Biosciences), anti-α-SMA (1:1,000; Sigma), and anti-type I collagen (1:1,000; Abcam) polyclonal antibodies at 4°C with gentle shaking overnight. Antibodies were detected by horseradish peroxidase-linked secondary antibody (Santa Cruz Biotechnology) using an Enhanced Chemiluminescence Western Blotting Detection System, in accordance with the manufacturer's instructions (Millipore, Billerica, MA, USA) [1]. The membrane was reblotted with anti-β-tubulin antibody (1:1,000; Applied Biological Materials Inc., Richmond, BC, Canada) to confirm equal protein loading in each lane. Transient transfection and reporter assays were performed as previously described [3].

Analysis of dipeptidyl peptidase-4 activity

Dipeptidyl peptidase-4 (DPP-4) activity was measured as described below. Plasma samples were separated by centrifugation (1,800×*g* for 20 minutes at 4°C) and used to measure DPP-4 activity. Kidney tissue was collected from the obstructed side and homogenized in 300 μL of ice-cold phosphate buffer solution. Then the supernatant obtained after centrifugation (20,000 ×*g* for 30 minutes at 4°C) was used to measure DPP-4 activity. DPP enzymatic activity of sCD26 was measured using the DPPIV-Glo™ Protease Assay (Promega, Mannheim, Germany), which is based on the cleavage of the substrate Gly-Pro-aminoluciferin by DPPIV. Light production produced by luciferase activity was used to calculate DPP enzyme activity. The assay was performed with 50 μL diluted sample and 50 μL freshly prepared CD26/DPPIV-Glo™ reagent for 30 minutes at room temperature in accordance with the manufacturer's protocol. Phosphate-buffered saline (50 μL) was used as a negative control.

Statistical analysis

All data are shown as the mean±standard error of the mean. Analysis of variance was used to compare multiple groups and $P<0.05$ was considered to represent statistical significance. All experiments were performed at least in triplicate.