Supplementary Data

Supplementary Materials and Methods

Preparation of conditioned medium

Rat adipose tissue-derived stem cells (ADSCs) and penile smooth muscle cells (PSMCs) were seeded in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) at 1×10^5 cells per well in 6-well culture plates. When 90% confluence was reached, the culture medium was removed and replaced with 1 mL of DMEM without FBS. Twenty-four hour later, the medium was harvested and centrifuged at 1,200 rpm for 10 min. The supernatant was recovered and stored at −80°C until use. Human ADSCs and PSMCs conditioned medium were prepared with the same protocol.

Culture of human umbilical vein endothelial cells, rat bladder smooth muscle cells, and urothelial cells

Human umbilical vein endothelial cells (HUVECs) were purchased from Lonza Inc. Then, 1.5×10^5 cells were seeded per well in 6-well culture plates. Afterward, the cells were treated in 4 different media: regular medium with normal glucose (5 mM, NG group), regular medium with high glucose (25 mM, HG group), human PSMCs conditioned medium with high glucose (HG + PSMCs CM group), and human ADSCs conditioned medium with high glucose (HG + ADSCs CM group) for 2 days. Rat bladder smooth muscle cells and urothelial cells were treated in the same way by using rat PSMCs or ADSCs conditioned medium.

Matrigel-based capillary-like tube formation assay

HUVECs were trypsinized, and 1.5×10^5 cells were seeded into 12-well culture plates that had been precoated with 300 μL of growth factor reduced matrigel (BD Biosciences). The cells of each group were maintained in their medium, respectively, and then incubated in a 37°C incubator with 5% CO_2 for 16 h to allow the formation of tubes. Endotubes were quantified by counting 3 random fields under the microscope (x100). Each condition was assessed in triplicate.

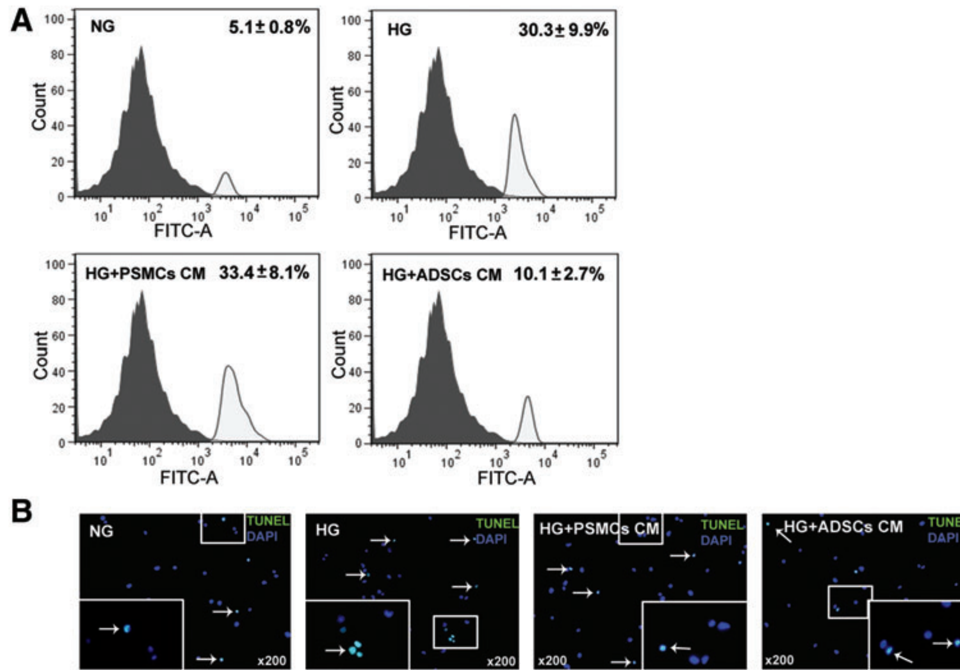
Determination of apoptosis in culture system

Rat bladder smooth muscle cells and urothelial cells that had just been cultured were detected for DNA damage by using TUNEL kit (BD Pharmingen) staining according to the instruction and counterstaining by DAPI. Afterward, the cells were photographed under a fluorescence microscope.

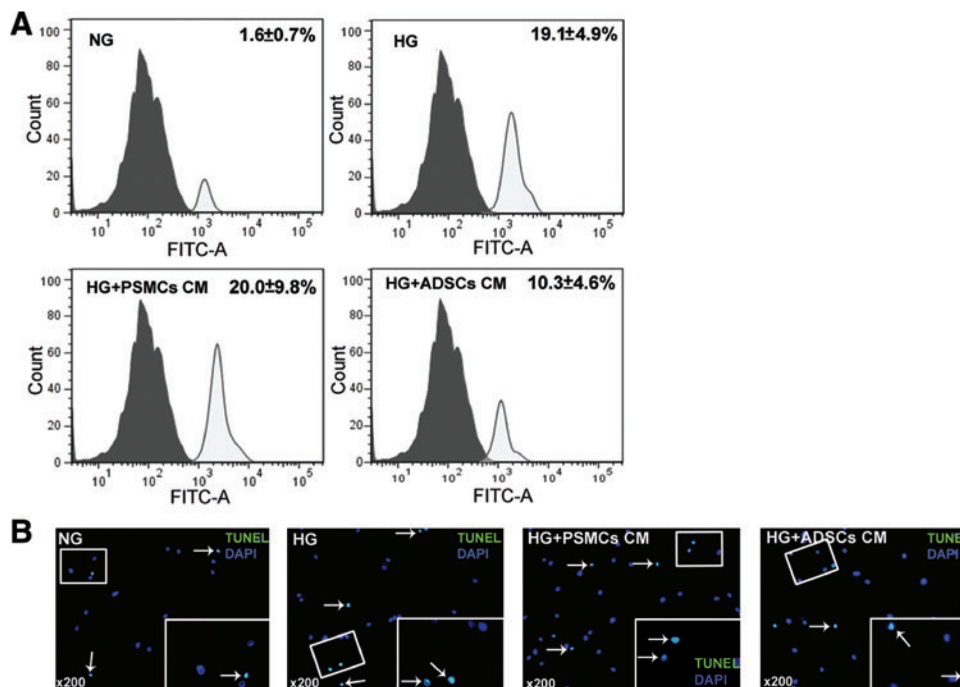
For quantification of apoptosis in cultured cells, flow cytometry was performed. The cells were suspended in 1% paraformaldehyde and washed twice with PBS. After fixation in 70% ice cold ethanol, the DNA of these cells was labeled with Apo-Direct Kit (BD Pharmingen). After washing, the resuspended cells were analyzed in a fluorescence-activated cell sorter (FACSVantage SE System; BD Biosciences). The percentage of apoptotic cells was determined. All data were analyzed with FlowJo software (Tree Star, Inc.). To generate statistically relevant data, each type of cell was tested in triplicate.

RNA isolation and real-time polymerase chain reaction

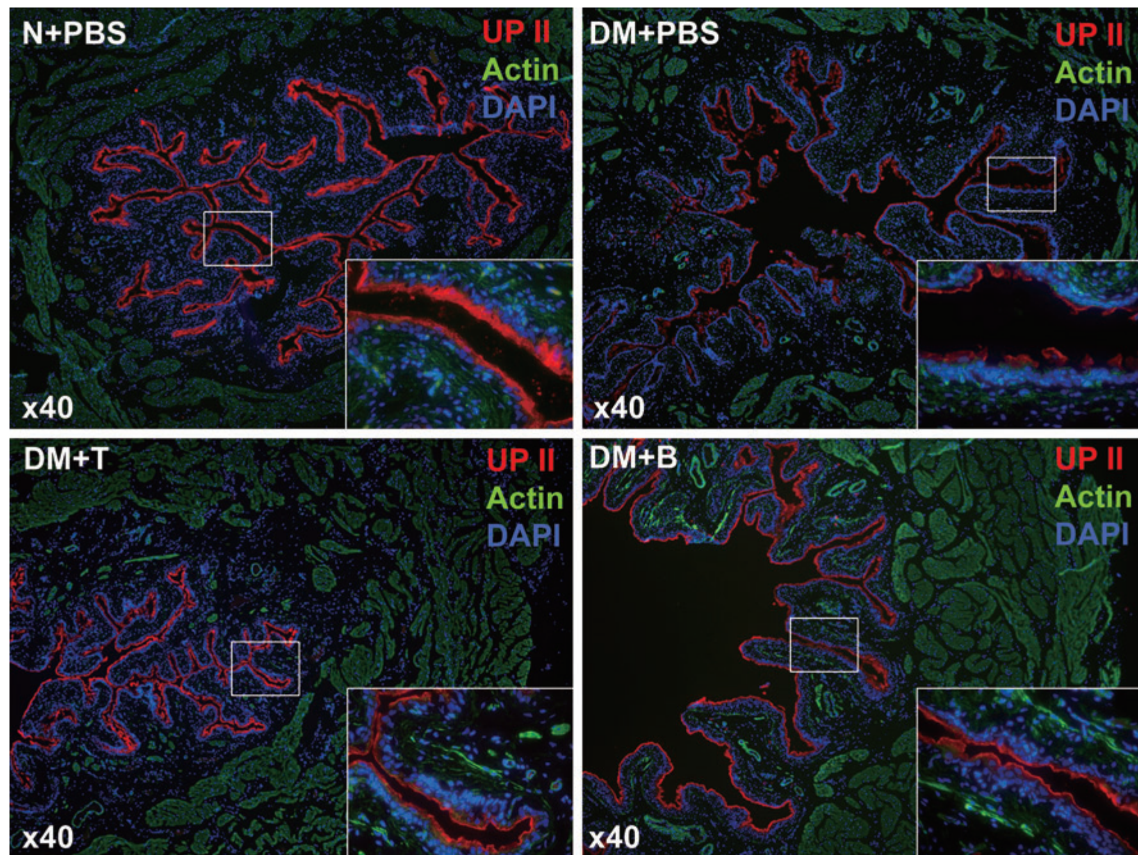
Total RNA from cultured rat bladder smooth muscle cells and urothelial cells was isolated by using RNAeasy Isolation Kit (Qiagen). Total RNA was reverse transcribed into a complementary DNA library. All reagents for real-time polymerase chain reaction (PCR), including the primers for rat Caspase-3 and glyceraldehydes-3- phosphate dehydrogenase (GAPDH), were purchased (Applied Biosystems). Primer sequences are presented as follows: Caspase-3 (Forward: 5′-AATTCAAGGGACG GGTCA-3′; Reverse: 5′-GCTT GTGCGCGTACAGTTTC-3′); GAPDH (Forward: 5′-ATGAT TCTACCCACGGCAAG-3′; Reverse: 5′-CTGGAAGATGGT GATGGGTT-3′). The reactions were run in Applied Biosystems' PRISM 7300HT sequence detection system using the 96-well plate format. The cycling conditions included an initial phase at 95°C for 3 min, 40 cycles at 95°C for 15 s, and 55°C for 1 min, followed by a melting curve analysis. The real-time PCR results were analyzed by Applied Biosystems' SDS 7000 software to determine the expression levels of Caspase-3 relative to those of GAPDH.



SUPPLEMENTARY FIG. S1. Apoptosis studies in cultured rat bladder urothelial cells. **(A)** Quantification of apoptosis in cultured urothelial cells was analyzed by flow cytometry. The data are presented as the fluorescence intensity (log scale) of apoptosis population (*light histograms*) and unstained control population (*dark histograms*). **(B)** TUNEL-positive cells could be observed in cultured rat bladder urothelial cells in vitro (*white arrow*). NG: regular medium with normal glucose. HG: regular medium with high glucose. HG + PSMCs CM: rat penile smooth muscle cells conditioned medium with high glucose. HG + ADSCs CM: rat ADSCs conditioned medium with high glucose. ADSC, adipose tissue-derived stem cell; PSMC, penile smooth muscle cell.



SUPPLEMENTARY FIG. S2. Apoptosis studies in cultured rat bladder smooth muscle cells. **(A)** Quantification of apoptosis in cultured smooth muscle cells was analyzed by flow cytometry. The data are presented as the fluorescence intensity (log scale) of apoptosis population (*light histograms*) and unstained control population (*dark histograms*). **(B)** TUNEL-positive cells could be observed in cultured cells in vitro (*white arrow*). NG: regular medium with normal glucose. HG: regular medium with high glucose. HG + PSMCs CM: rat penile smooth muscle cells conditioned medium with high glucose. HG + ADSCs CM: rat ADSCs conditioned medium with high glucose.



SUPPLEMENTARY FIG. S3. UP II was normally distributed in a dense layer concentrated on the luminal surface of the umbrella cells. In the diabetic state, it lost continuity, abruptly disappeared, or became thin in some areas. The expression of UP II was improved after ADSCs injection. UP II, uroplakin II.