Supplementary Experimental Procedures

TGF-β induced ovarian cancer cell motility in the presence of ovarian fibroblasts

NOF151-hTERT fibroblasts were seeded onto a 24-well culture plate at a density of 5×10^4 cells per well. After the cells were seeded, culture medium was aspirated and the cells were washed with phosphate-buffered saline (PBS). Serum-free medium supplemented with 1 ng/mL epidermal growth factor (EGF) and with or without 10 μg/mL polyclonal TGF-β-neutralizing antibody raised against recombinant and platelet-derived TGF-β from multiple species (R&D Systems) was added to the experimental and control wells. According to the manufacturer's product specifications, the neutralization dose (ND_{50}) range of the antibody is approximately 5-30 μg/mL as determined according to its ability to neutralize TGF-β1 inhibition of IL-4-dependent proliferation in the HT-2 mouse T cell line. High-grade serous ovarian cancer cell lines ALST, HeyA8, and OVCA433 were resuspended in serum-free RPMI medium and seeded onto 0.4-μm-pore 24-well cell inserts (BD Biosciences) at 5×10^4 cells per insert and co-cultured with the prepared NOF151-hTERT cells for 48 hours. After incubation, the inserts were removed and replaced with 8-μm-pore 24-well inserts (BD Biosciences) seeded with freshly trypsinized ALST, HeyA8, and OVCA433 cells in the corresponding wells that were allowed to migrate for 15 hours. The cells were then stained with calcein AM (Life Technologies), and the number of migrated cells in each chamber in images of nine random fields of view per insert obtained via fluorescence microscopy were quantified using the Image-Pro Plus software program (version 7.0; Media Cybernetics).

Similar co-culture system was employed to further evaluate the effect of

exogenous TGF-β on induction of ovarian cancer cell motility in the presence of ovarian fibroblasts. NOF151-hTERT cells were seeded onto 24-well plates at a density of 5 × 10^4 cells per well. The culture medium was removed and the cells were washed with PBS the next day. Serum-free medium supplemented with 1 ng/mL EGF or serum-free medium with EGF supplemented with TGF-β1 or TGF-β2 at the concentration of 5 ng/mL or 10 ng/mL was added to each control and experimental well. 48 hours later, ALST or HeyA8 cells seeded onto 8-μm-pore 24-well inserts in a serum-free medium were co-cultured with NOF151-hTERT cells in the control and experimental wells and allowed to migrate for 15 hours. The cells were then stained with calcein AM, and the number of migrated cells in each chamber in images of nine random fields of view per insert obtained via fluorescence microscopy were quantified using the Image-Pro Plus software program.

Determination of the TGF-β concentration in culture medium using ELISA

A similar co-culture system was set up as described above. ALST cells resuspended in serum-free RPMI medium were seeded onto 0.4-μm-pore 12-well cell inserts (BD Biosciences) at 5 × 10^4 cells per insert and co-cultured with the prepared NOF151-hTERT cells for 48 hours in the presence or absence of TGF-β-neutralizing antibody. Cell culture medium was collected from the culture system after 24 and 48 hours and the concentrations of TGF-β1 and TGF-β2 were determined using the TGF-β1 ELISA kit (Life Technologies) and the human TGF-β2 Quantikine ELISA kit (R&D Systems), respectively. Measurements of TGF-β concentration were conducted in triplicate, and the mean values are presented.

Transcriptome profiling of TGF-β treated fibroblasts and VCAN-treated ovarian cancer cells

RNAs were isolated from NOF151-hTERT fibroblasts treated with PBS, TGF-β1 or TGF-β2, and from OVCA433 ovarian cancer cells treated with VCAN-conditioned medium and control conditioned medium from three independent sets of experiments. One hundred nanograms of RNA from each sample was used to generate biotin-labeled aRNA using the MessageAmp Permier RNA Amplification Kit (Life Technologies), which was then subjected to whole-genome transcriptome profiling using GeneChip Human Genome U133 Plus 2.0 microarrays (Affymetrix). Differentially expressed genes with more than twofold change in expression when compared to the corresponding controls and with (*P* values smaller than 0.05) were selected for further analysis.

Enhancement of ovarian cancer cell motility by TGF-β mediated via upregulation of VCAN expression in neighboring stromal fibroblasts

To confirm that TGF-β-induced increases in ovarian cancer cell motility were *VCAN*-dependent, endogenous VCAN in fibroblasts was knocked down by *VCAN*-specific siRNA (Life Technologies) or neutralized by a polyclonal anti-VCAN antibody (Seikagaku Corporation). Following VCAN knockdown or neutralization, co-culture experiment with NOF151-hTERT fibroblasts and ovarian cancer cells were set up and motility assay of the cancer cells were performed as described above.

Effect of exogenous VCAN on ovarian cancer cell motility

Oris cell migration assay (Platypus Technologies) was used with seven high-grade serous ovarian cancer cell lines (ALST, HeyA8, OVCA3, OVCA420, OVCA429, OVCA433, and SKOV3) treated with VCAN-positive or -negative conditioned medium prepared from CHO cell lines according to the manufacturer's protocol. In brief, ovarian cancer cells were seeded and adhered on a 96-well plate in the presence of rubber stoppers. The stoppers were then removed to create a cell-free migration zone. Next, the cells were treated with VCAN-positive or -negative conditioned medium and allowed to migrate for 24 hours. At the end of the experiment, cells were stained with calcein AM, and their migration was quantified by analyzing fluorescent images using the ImageJ software program (National Institutes of Health).

VCAN-stimulated ovarian cancer cell invasion

The invasion potential of ovarian cancer cells under the effect of VCAN was evaluated using a Matrigel invasion assay with the BD BioCoat Matrigel invasion chamber (BD Biosciences) according to the manufacturer's protocol. In brief, 3×10^4 ovarian cancer cells (ALST, HeyA8, or OVCA433) were seeded onto an 8-μm BD BioCoat Matrigel invasion chamber in serum-free medium and inserted into a companion plate with VCAN-conditioned medium. After incubation for 15 hours, cells were stained with calcein AM, and non-invading cells were removed from the upper surface of the membrane via scrubbing with cotton-tipped swabs. The invading cells were counted by photographing the membrane via fluorescent microscopy and analyzing the photographs using the Image-Pro Plus software program. To confirm the

effect of VCAN on ovarian cancer cell invasion in the 3D culture model, 1×10^5 HeyA8 cells were embedded in a 0.2% type I collagen matrix prepared with PBS, Opti-MEM (Life Technologies), FBS, and sodium bicarbonate with or without VCAN-conditioned medium. Cell invasion was then monitored using confocal microscopy and live cell imaging at 37°C in a 5% CO_2-containing atmosphere for 48 hours.

TGF-β-induced VCAN expression in ovarian fibroblasts mediated by the SMAD signaling pathway

To determine whether induction of *VCAN* in ovarian fibroblasts by TGF-β is mediated via the TGF-β receptors and SMAD signaling, the effect of TGF-β on this induction in NOF151-hTERT fibroblasts was evaluated in the presence of TGF-β receptor/SMAD pathway inhibitors by seeding 1×10^5 NOF151-hTERT cells onto each well of a 12-well plate. Serum-free MCDB 105/199 medium with EGF in the presence or absence of TGF-β receptor type 2 inhibitors, ALK5 inhibitors I and II, or the SMAD3-specific inhibitor SIS3 was added to the cells. After pre-treating NOF151-hTERT cells with inhibitors for 6 hours, TGF-β was added to the fibroblasts to induce VCAN expression. After 48 hours of incubation, total RNA was isolated from NOF151-hTERT cells in the control and experimental groups. The high-capacity cDNA reverse transcription kit (Life Technologies) was used to synthesize cDNA from mRNA. *VCAN* expression levels in different experimental groups were assayed using qRT-PCR with a pre-designed TaqMan probe (Life Technologies) and SsoFast Probes Supermix (Bio-Rad Laboratories).

Luciferase reporter assay

The OVCA429 and OVCA433 cell lines were stably transduced with different reporter response elements from the Cignal Lenti Reporter system (SABiosciences). The cells were plated at a density of 3 x 10^4 cells per well in 96-well plates and allowed to attach for 24 hours. The medium was then replaced with either VCAN-conditioned medium or control conditioned medium and incubated for 12 hours. Cells were then lysed with luciferase cell lysis buffer and luciferase activity was assayed by the Luciferase Assay System (Promega) according to the manufacturer's instructions using a FLUOstar Omega plate reader (BMG Labtech).

qRT-PCR analysis

The 2-ΔΔCT method was used to calculate the relative expression of genes. Pre-designed human TGF-β receptor type 1 (Hs00610320_m1), TGF-β receptor type 2 (Hs00234253_m1), *POSTN* (Hs00170815_m1), *COMP* (Hs00164359_m1), *VCAN* (Hs00171642_m1), *DACT1* (Hs00420410_m1), *CD44* (Hs01075861_m1), *HMMR* (Hs00234864_m1), and *MMP9* (Hs00234579_m1) TaqMan gene expression assays (Life Technologies) were used in qRT-PCR for expression analysis.

Western blot analysis

Western blot analysis was performed to measure VCAN, CD44, HMMR, and MMP9 protein expression. For detection of VCAN in NOF151-hTERT cells using commercially available anti-VCAN antibody (Sigma-Aldrich), the total cell lysate was harvested from TGF-β-treated NOF151-hTERT fibroblasts, and conditioned medium

was collected and concentrated by 50-fold using a Centricon concentrator with a 100-kDa cut-off (Millipore). Cell lysates and concentrated conditioned media from control and treatment samples were digested using chondroitinase ABC (Sigma-Aldrich) for 3 hours at 37°C before being loaded into polyacrylamide gels for Western blot analysis. CD44, HMMR, and MMP9 were detected in lysates of control and VCAN-treated ovarian cells using commercially available anti-CD44 (Sigma-Aldrich), anti-HMMR (Epitomics), and anti-MMP9 (Cell Signaling Technology) antibodies.

Immunohistochemistry

Immunolocalization of TGF-β receptor types 1 and 2 was performed with 15 FFPE high-grade serous ovarian tumor sections and 8 normal ovary tissue sections, whereas immunolocalization of VCAN, CD44, HMMR, and MMP9 was performed with 45 FFPE ovarian tumor tissue sections obtained from high-grade serous ovarian cancer patients. The tissue samples were obtained from the Ovarian Cancer Repository under protocols approved by the MD Anderson Institutional Review Board. Tissue slides were stained with commercially available anti-TGF-β receptor type 1 (1:100; Cat# SAB2700824, Sigma-Aldrich), anti-TGF-β receptor type 2 (1:100; Cat# NB100-91996, Novus Biologicals), anti-VCAN (1:200; Cat# HPA004726, Sigma-Aldrich), anti-CD44 (1:200; Cat# SAB1405590, Sigma-Aldrich), anti-HMMR (1:100; Cat# PAB19602, Abnova), and anti-MMP9 (1:200; Cat# 3852, Cell Signaling Technology) antibodies. Target protein expression was visualized using either the Warp Red chromogen kit or the Bzoid DAB chromogen kit (Biocare Medical).