Supplementary Materials and Methods

RNA isolation, reverse transcription, and quantitative real-time PCR

Total RNA was extracted using Trizol (Invitrogen; Carlsbad, California). To quantify the expression of TGF-β1, we subjected the total RNA to polyadenylation and reverse transcription (RT) using a ThermoScript^{TM} RT-PCR System (Invitrogen). Real-time polymerase chain reaction (PCR) analysis was carried out using an SYBR Green PCR master mix (Applied Biosystems; Foster City, California) on an ABI 7500HT system. GAPDH snRNA was used as an endogenous control. All samples were normalized to internal controls, and fold changes were calculated through relative quantification ($2^{-\Delta\Delta CT}$). Real-time PCR for target genes was performed as previously described [1]. The primers used are shown in Supplementary Table S2.

Western blot analysis

Protein expression was assessed by immunoblot analysis of cell lysates (20-60 μg) in RIPA buffer in the presence of rabbit antibodies to E-cadherin (Cat# 3195, RRID: AB_2291471), N-cadherin (Cat# 13116, RRID:AB_2687616), β-catenin (Cat# 8480, RRID:AB_11127855), vimentin (Cat# 5741, RRID:AB_10695459), p21 (Cat# 2947, RRID:AB_823586), Snail (Cat# 3879, RRID:AB_2255011), Smad2 (Cat# 5339, RRID:AB_10626777), phosphorylation-Smad2 (p-Smad2) (Cat# 18338, RRID:AB_2798798); mouse antibodies to human runt-related transcription 3 (RUNX3) (Cat# 13089, RRID:AB_2798118) and GAPDH (Cat# 51332, RRID:AB_2799390) (1:1000; Cell Signaling Technology; Danvers, Massachusetts); rabbit antibodies to TGF-β1 (Cat# BS91338), fibronectin, RUNX3 (Cat# BS8826) and histone H3(K4) (Cat# BS1174) protein (1:500; Bioworld; Minneapolis, Minnesota). The membranes, probed with the indicated primary antibodies, were subjected to the appropriate horseradish peroxidase-conjugated secondary antibodies

(anti-rabbit: Cat# 7074, RRID:AB_2099233 and anti-mouse: Cat# 7076, RRID:AB_330924), and developed by enhanced chemiluminescence.

Cell migration/invasion analysis

Cells were maintained in serum-containing growth medium with treatments of ethanol (0, 100, 200 mg/dl) and/or 4-MP, PFD, vectors for 48-72 h, and maintained in serum-free medium for 24 h. Cells (1×10^5 cells/100 μl) were added to 8-μm pore Transwell and Matrigel chamber plates respectively (Corning Star, Cambridge, Massachusetts). The bottom chamber was prepared using 10% fetal bovine serums as a chemoattractant. Cells were allowed to migrate through the porous membrane or matrigel for 48 h and 72 h respectively at 37□. The cells that stuck to the lower surface of the membrane were treated with a fixation/staining solution (0.1% crystal violet, 1% formalin, and 20% ethanol) for visualization. The cells were counted under a microscope (in 5 randomly selected fields; original magnification: ×200). At least five chambers from three different experiments were analyzed.

Cell motility assay

Confluent monolayers of cells were maintained in serum-containing growth medium with treatments of ethanol (0, 100, 200 mg/dl) and/or 4-MP, PFD, vectors for 48-72 h, and maintained in serum-free medium for 24 h. A 1000-μl plastic pipette tip was used to scratch the monolayers. The wounded cells were then cultured in serum-free medium for an additional 48 h and photographed under an inverted phase contrast

microscope. Three different points were marked on the plate, and the distance between each point and the edge of the scratch wound was measured before and after cell migration. The mean migration distance (μm) was calculated by subtracting the length after 48 h from that at 0 h. The result was expressed as a migration index, i.e., the distance migrated by treated cells compared with the distance migrated by control cells. Experiments were carried out in triplicate and repeated at least five times.

Immunofluorescence assay

Cell were culture on coverslips overnight, fixed with 4% paraformaldehyde for 30 min, and then treated with 0.5% Triton X-100 for 15 min. After being blocked in 10% normal blocking serum at room temperature for 15 min, the slides were incubated with antibodies from Cell Signaling Technology (USA), including rabbit antibodies to E-cadherin (Cat# 3195, RRID: AB_2291471), Vimentin (Cat# 5741, RRID:AB_10695459), Smad2 (Cat# 5339, RRID:AB_10626777), phosphorylation-Smad2 (p-Smad2) (Cat# 18338, RRID:AB_2798798), mouse antibody to RUNX3 (Cat# 13089, RRID:AB_2798118) (1:150) at 4℃ overnight. Then followed by thrice phosphate-buffered saline (PBS) rinsing. Cover slips were incubated with antibodies from Cell Signaling Technology (USA), including a fluorescein isothiocyanate (FITC)-conjugated anti-rabbit (Cat# 4412, RRID:AB_1904025) or mouse (Cat# 4408, RRID:AB_10694704) stain and Texas Red-conjugated anti-mouse (Cat# 8890, RRID:AB_2714182) or –rabbit antibodies (Cat# 8889, RRID:AB_2716249)(1:200) for 30 min at room temperature, then stained

with 6-diamidino-2-phenylindole (DAPI, Cat# D3571, Invitrogen, USA).

Enzyme-linked immunosorbent assay

The supernatants and cytoplasm from SW480 and HCT116 cells were collected. The supernatant was used to measure the total levels of extracellular TGF-β1, while the cytoplasm was use to measure the total level of intracellular TGF-β1, by using the human TGF-β1 ELISA Kit (Cat# ml001546, Enzyme-linked Biotechnology, Shanghai, China) according to the manufacturer's introductions. The cytokine expression level (pg/ml) per 10^5 cells was analyzed.

Co-immunoprecipitation assay

After ethanol treated for 48-72 h, cells were harvested and washed three times with chilled PBS, then lysed in IP Lysis Buffer (Pierce, USA) with Protease and Phosphatase Inhibitor Cocktail (Sigma, USA). The protein was quantified by using a BCA Protein Assay Kit (KeyGen, Biotechnology, China), then diluted into 1 mg/ml with chilled wash buffer (with protease and phosphatase inhibitor), and divided into two equal parts, which were added with rabbit anti-Smad2 (Cat# 5339, RRID:AB_10626777, Cell Signaling Technology, USA) or p-Smad2 antibody (Cat# 18338, RRID:AB_2798798, Cell Signaling Technology, USA) (3-4μg) and the same amount of rabbit IgG (Beyotime, China) respectively, rotating slowly, and incubated overnight at 4℃. Protein A agarose beads were washed twice with cold wash buffer followed by adding them into the two parts of protein based on 20 μl every 1 ml total

protein, then incubated for 4-6 h at 4℃ with low-speed rotation. Afterwards, the protein mixture was washed with 500 μl chilled wash buffer (containing protease and phosphatase inhibitor) for three times. Dilute all the protein mixtures with wash buffer. After heat denaturation in 10% SDS-PAGE sample loading buffer, the protein samples were subjected to western blot assay.

Mouse tail-vein assay

To determine the lung homing potential of cancer cells *in vivo*, we injected 5×10^6 ethanol-treated cells and LV-control into nude mice (*N*=6/group) through the tail vein. Whole-body optical images were obtained to monitor primary tumor growth and the formation of metastasis lesions. The mice were all sacrificed 8 weeks later, at which time individual organs were removed and metastatic tissue was analyzed using hematoxylin and eosin stains.

Referrence:

1. Wang H, An H, Wang B, Liao Q, Li W, Jin X*, et al.* miR-133a represses tumour growth and metastasis in colorectal cancer by targeting LIM and SH3 protein 1 and inhibiting the MAPK pathway. *European journal of cancer* 2013, 49(18): 3924-3935.