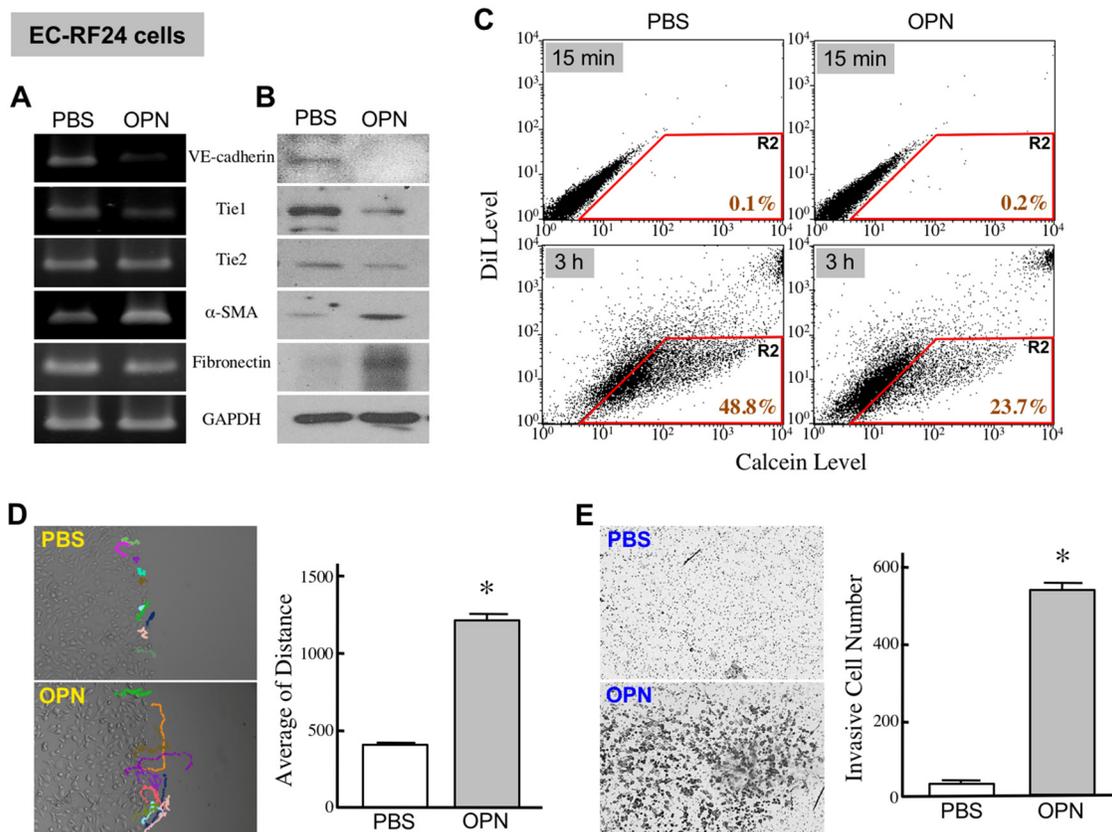
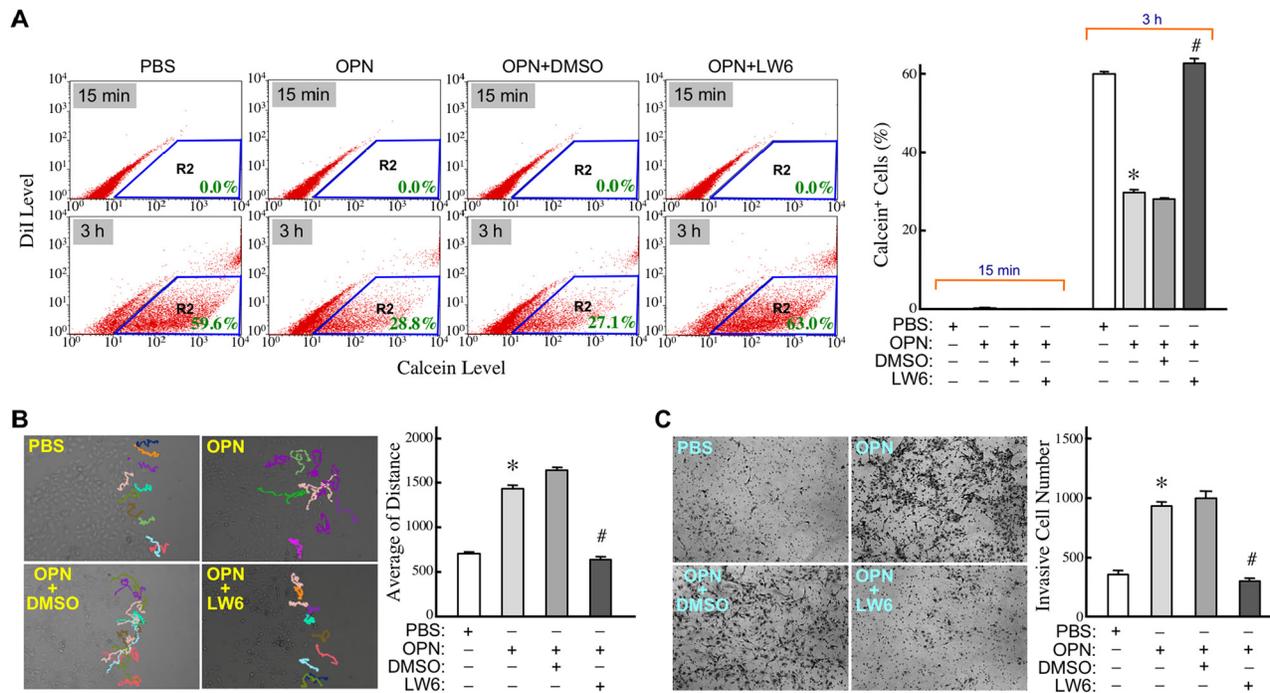


Osteopontin-integrin engagement induces HIF-1 α -TCF12-mediated endothelial-mesenchymal transition to exacerbate colorectal cancer

SUPPLEMENTARY MATERIALS



Supplementary Figure 1: OPN induces EndoMT in EC-RF-24 cells, an immortalized EC line. (A) mRNA levels of VE-cadherin, Tie1, Tie2, α -SMA, and fibronectin in EC-RF-24 cells pre-incubated 16 h with 2% FBS-containing medium and then treated with PBS or 0.3 μ g/ml of OPN for another 15 h. (B) VE-cadherin, Tie1, Tie2, α -SMA, and fibronectin levels in EC-RF-24 cells pre-incubated 16 h with 2% FBS-containing medium and then treated 24 h with PBS or 0.3 μ g/ml of OPN. It was noted that OPN changed the protein levels of Tie2 and fibronectin but did not significantly affect their mRNA levels. (C) Gap-junction activities in EC-RF24 cells treated with PBS or OPN as described in (B). Representative dot plots from 3 independent Calcein-transfer assays are shown. The cells in the R2 regions were categorized as Calcein-accepting cells, and the % of Calcein-accepting cells represented cellular gap-junction activities of the tested cells. (D) Cell migration activities in EC-RF-24 cells treated with PBS or OPN as described in Methods. Cell migration was monitored for 15 h using time-lapse photography, and the movement tracks of 10 randomly selected PBS or OPN-treated EC-RF-24 cells were analyzed by Image-Pro Plus software. Quantification of the accumulated migration distances is shown in the right panel. The mean \pm SD values of 3 independent experiments are shown. *, $P < 0.05$ when compared with PBS-treated cells. (E) Invasiveness of PBS or OPN-treated EC-RF-24 cells determined by Transwell invasion assay. The representative images shown were invasive cells on the filters of Transwell inserts. The mean \pm SD values of 3 independent experiments are shown in the right panel. *, $P < 0.05$ when compared with PBS-treated cells.



Supplementary Figure 2: Antagonistic effect of LW6 on OPN-inhibited cellular gap-junction as well as OPN-induced cell migration and invasion in HUVECs. Assays of cellular gap-junction (A), migration (B), and invasion (C) activities in HUVECs treated as described previously with PBS, OPN, or OPN together with DMSO or LW6. In each panel, the left side is the representative plots/ images, and the quantitative data shown in the right side are mean \pm SD values of 3 independent experiments. *, $P < 0.05$ when compared with PBS-treated cells. #, $P < 0.05$ when compared with the treatment with OPN plus DMSO.

Supplementary Table 1: Microwestern array assay of protein levels in Endo CM vs. EndoMT CM

See Supplementary File 1

Supplementary Table 2: RT-PCR analyses of mRNA levels of the following genes. The primers and PCR conditions were listed below. All reactions started at 95°C for 5 min and terminated at 72°C for 7 min

Gene	Primer Sequence	PCR Condition & Product Size
VE-cadherin	Forward: 5'-GTT-TCG-TGG-TGT-TAT-GTC-CT-3' Reverse: 5'-AGT-TGT-TCC-GAG-TCA-CAA-AA-3'	95°C (30 sec), 49°C (40 sec), and 72°C (40 sec) for 32 cycles; 243 bp
Tie1	Forward: 5'-AGT-TTC-GAG-GCT-GCT-CCA-3' Reverse: 5'-TGT-GCT-GGT-CGG-AGA-GAA-3'	95°C (30 sec), 53°C (40 sec), and 72°C (40 sec) for 25 cycles; 282 bp
Tie2	Forward: 5'-GGC-AAG-AAG-GAA-CAG-CAG-A-3' Reverse: 5'-GCA-AAA-GCA-GCA-GCA-GAA-G-3'	95°C (30 sec), 53°C (40 sec), and 72°C (40 sec) for 25 cycles; 267 bp
CD31	Forward: 5'-TGT-CTC-CAG-ACA-CCA-TTC-CA-3' Reverse: 5'-TTA-GAG-CGC-CCT-CTT-GTG-TT-3'	95°C (30 sec), 52°C (45 sec), and 72°C (45 sec) for 25 cycles; 202 bp
α-SMA	Forward: 5'-TCC-AGA-GGC-ATA-GAG-AGA-CA-3' Reverse: 5'-ACC-CTG-AAG-TAC-CCG-ATA-GA-3'	95°C (30 sec), 52°C (40 sec), and 72°C (40 sec) for 30 cycles; 222 bp
Fibronectin	Forward: 5'-ACC-AAC-CTA-CGG-ATG-ACT-CG-3' Reverse: 5'-GCT-CAT-CAT-CTG-GCC-ATT-TT-3'	95°C (30 sec), 56°C (40 sec), and 72°C (40 sec) for 33 cycles; 229 bp
TCF12	Forward: 5'-GTC-GAT-TAG-GAG-CCC-ATG-AA-3' Reverse: 5'-TAT-GCT-GTC-CCA-GGT-TTT-CC-3'	95°C (30 sec), 56°C (40 sec), and 72°C (40 sec) for 25 cycles; 202 bp
Twist-1	Forward: 5'-GGC-CGG-AGA-CCT-AGG-TAA-GG-3' Reverse: 5'-CAT-CCC-CCT-GGA-GAC-TGC-3'	95°C (30 sec), 58°C (40 sec), and 72°C (40 sec) for 30 cycles; 218 bp
Snail	Forward: 5'-CAG-ACC-CAC-TCA-GAT-GTC-AA-3' Reverse: 5'-CAT-AGT-TAG-TCA-CAC-CTC-GT-3'	95°C (45 sec), 53°C (45 sec), and 72°C (45 sec) for 30 cycles; 557 bp
HIF-1α	Forward: 5'-CAG-CTA-TTT-GCG-TGT-GAG-GA-3' Reverse: 5'-CCA-AGC-AGG-TCA-TAG-GTG-GT-3'	95°C (30 sec), 62°C (1 min), and 72°C (1 min) for 30 cycles; 470 bp
CD133	Forward: 5'-ATC-CAT-CCT-ATC-CTG-GAA-GA-3' Reverse: 5'-CCT-GTC-TGT-CTC-TTG-CAC-TC-3'	95°C (30 sec), 56°C (30 sec), and 72°C (1 min) for 30 cycles; 264 bp
ALDH1	Forward: 5'-CAA-GCA-GAC-ATG-ACA-TCC-TAG-G-3' Reverse: 5'-TCC-TTT-GTA-GCC-TAC-TTT-TGC-A-3'	95°C (30 sec), 56°C (30 sec), and 72°C (1 min) for 30 cycles; 189 bp
CD44	Forward: 5'-GCA-GAA-TGT-GGA-CAT-GAA-GA-3' Reverse: 5'-ATG-CTA-AAA-AAG-ATT-CGC-AAT-G-3'	95°C (30 sec), 56°C (30 sec), and 72°C (1 min) for 30 cycles; 150 bp
CD24	Forward: 5'-GGT-GCC-TGT-AAT-CCC-AGC-TA-3' Reverse: 5'-CTC-CTG-AGG-CTT-TGG-ATT-TG-3'	95°C (30 sec), 56°C (30 sec), and 72°C (1 min) for 30 cycles; 394 bp
CD326	Forward: 5'-ATA-GAA-GAA-GGG-AAA-TAG-CAA-ATG-G-3' Reverse: 5'-TTA-ACG-ATG-GAG-TCC-AAG-TTC-TGG-3'	95°C (30 sec), 56°C (30 sec), and 72°C (1 min) for 30 cycles; 232 bp
GAPDH	Forward: 5'-GAA-GGT-GAA-GGT-CGG-AGT-3' Reverse: 5'-GAA-GAT-GGT-GAT-GGG-ATT-TC-3'	95°C (30 sec), 56°C (40 sec), and 72°C (40 sec) for 25 cycles; 220 bp