**Supplementary Materials and Methods**

**RNA isolation, reverse transcription, and PCR**

Total RNA was extracted by Trizol reagent (Invitrogen) according to the manufacturer's instructions. Reverse transcription (RT) reactions contained 2 µg of RNA, 50 nmol/L stem-loop RT primer, 0.25 mmol/L each deoxynucleotide triphosphate, 50 units of Moloney murine leukemia virus reverse transcriptase (Invitrogen), 1× RT buffer, 10 mmol/L DTT, and 4 units of RNase inhibitor. The stem-loop RT primers were designed according to Chen and Ridzon et al ([1](#_ENREF_1)). The mature miRNA sequences were obtained from the Sanger Center miRNA Registry (<http://microrna.sanger.ac.uk/sequences/>). The reactions were incubated at 37°C for 50 minutes, 16°C for 30 minutes, followed by pulsed RT reaction: 60 cycles at 20°C for 30 seconds, 42°C for 30 seconds, and 50°C for 1 second. The reaction was terminated by 5 min incubation at 85°C to inactivate the reverse transcriptase enzyme. In addition to detect mRNA levels, 5 μg aliquot of total RNA was subjected to a RT-PCR reverse transcriptase kit (Invitrogen). The cDNA products were diluted with ddH2O. Equal amount of cDNA (2 μL) was used to perform PCR. After initial denaturation at 94°C for 4.5 minutes, cycling parameters were as follows: *VEGF-C*, denaturation (94°C, 1 minute), annealing (53°C, 1 minute), and extension (72°C, 1 minute); the reaction included 28 cycles; *CTTN*, denaturation (94°C, 1 minute), annealing (55°C, 1 minute), and extension (72°C, 1 minute); the reaction included 30 cycles; *Ago2*, denaturation (94°C, 1 minute), annealing (52°C, 30 seconds), and extension (72°C, 1 minute); the reaction included 28 cycles; *GAPDH*, denaturation (94°C, 30 seconds), annealing (58°C, 30 seconds), and extension (72°C, 30 seconds); the reaction included 22 cycles.

**Western blot**

ESCC cells were lysed in NETN lysis buffer (20 mmol/L Tris-HCl, pH 8.0, 150 mmol/L NaCl, 0.5% Nonidet P-40 and 1 mmol/L EDTA) containing protease inhibitor cocktail (Sigma-Aldrich) for 5 min with sonication and then centrifuged at 16,000 × g for 30 minutes. An equal quantity of protein from cell lysates was resuspended in gel sample buffer, resolved by 10% SDS-polyacrylamide gel electrophoresis, and transferred to PVDF membranes as described previously ([2](#_ENREF_2)). After blocking, blots were incubated with specific primary antibodies, and after washing and incubating with secondary antibodies, immunoreactive proteins were visualized using an enhanced chemiluminescence detection system (PerkinElmer).

**shRNA expression and lentivirus production**

For establishment of VEGF-C silenced cells, shVEGF-C sequence cloned in the pGIPZ plasmids were provided by Dr. Michael Hsiao’s Lab as kindly gifts. For establishment of CTTN-silenced cells, shCTTN plasmids were purchased from National RNAi Core Facility, Academia Sinica, Taipei, Taiwan. These respective plasmids, pCMV-deltaR8.91 and pCMV-VSV-G were cotransfected into HEK293T cells 48 hours to produce lentivirus which would be collected and applied for infection. TE-8 cells were then infected with lentivirus, selected with puromycin (5 µg/mL).

**Real-time PCR quantification**

Real-time PCR was performed using a Roche LightCycler 480 Real-Time PCR system (Roche). For miRNA level detection, Real-time PCR reactions contained 0.5 μmol/L forward and reverse primers, 1 μmol/L Universal ProbeLibrary Probe #21 (Roche), 1× LightCycler TaqMan Master mix, and 2 μL of cDNA. Amplification curves were generated with an initial denaturing step at 95°C for 10 minutes, followed by 65 cycles of 95°C for 5 seconds, 60°C for 10 seconds, and 72°C for 1 second. The U47 small nuclear RNAs were used as an internal control. The reverse primer for all above genes was 5′-GTGCAGGGTCCGAGGT-3′. For mRNA level detection, PCR reactions contained 0.5 μmol/L of each forward and reverse primer, 1 μmol/L Universal ProbeLibrary Probe (Roche), 1× LightCycler TaqMan Master mix, and 2 μL of cDNA. Amplification curves were generated with an initial denaturing step at 95°C for 10 minutes, followed by 55 cycles of 95°C for 5 seconds, 60°C for 10 seconds, and 72°C for 1 second. The *GAPDH* were used as an internal control.

**Cell tracing assay**

5 × 104 cells were plated in the 6 cm dish the day prior to performing the cell tracing assay. Original cultured media was changed to serum-free media with or without rhVEGF-C. The cells were then placed into the incubation container at 37°C in a humidified atmosphere at 5% CO2 which is placed on the stage of a light microscope (Zeiss). Seventy-five pictures of living cells were taken every 15 minutes at 50× magnification. To analyze these data, Image J (NIH), was used to count and track cells according to manufacturer instructions. We took at least twenty single cells to calculate the whole path distance to represent the migration ability of these cells.

**Supplementary Figure Legend**

**Supplementary Figure S1.** The mRNA expression of *Nrp1 and Nrp2* in ESCC cell lines. RT-PCR was showed the expression level of *Nrp1 and Nrp2* in TE1, TE12, TE3, TE7, and TE8 cells. *GAPDH* was used as an internal control.

**Supplementary Figure S2.** The phosphorylated VEGFR-3 in TE-1 cells with VEGF-C and VEGF-A treatment. TE-1 cells were starved with serum-free medium for 16 hours and treated with 100 and 200 ng/mL VEGF-C as well as 200 ng/mL VEGF-A for 10 minutes, following examination of phosphorylated VEGFR-3 (p-VEGFR-3, Y1063/1068) expression by Western blot. β-actin was used as an internal control.

**Supplementary Figure S3.** The mRNA expression of *CTTN* in rhVEGF-C-treated TE-1 cells. TE-1 cells were treated with a various doses of rhVEGF-C (0, 25, 50, 100 and 200 ng/mL) for 24 hours and isolated total RNA to measure the expression level of *CTTN* by RT-PCR. *GAPDH* was used as an internal control.

**Supplementary Figure S4.** The mRNA expression of *miR-326* in TE-8 cells with knockdown of VEGFR-3. TE-8 cells were transiently transfected shVEGFR-3#1, #2 or shCtrl for 48 hours and isolated total RNA to assay. A, qRT-PCR was used to quantify the expression level of miR-326 in those indicated cells. B, RT-PCR was showed the expression level of *VEGFR-3* in those indicated cells. *GAPDH* was used as an internal control. The quantities values were evaluated as percentage compared with TE-8/shCtrl cells. The columns are the mean values from three independent experiments. Bars indicate means ± SE.

**Supplementary Figure S5.** The mRNA expression of *Dicer* between TE-1/VEGF-C and TE-1/vector cells. TE-1 cells were stably expressed VEGF-C or vector control and isolated total RNA to assay. qRT-PCR was used to quantify the expression level of *Dicer*, and the quantities values were evaluated as percentage compared with TE-1/vector cells. The columns are the mean values from three independent experiments. Bars indicate means ± SE.

**References**

1. Chen C, Ridzon DA, Broomer AJ, Zhou Z, Lee DH, Nguyen JT, et al. Real-time quantification of microRNAs by stem-loop RT-PCR. Nucleic Acids Res 2005;33:e179.

2. Yu YL, Su KJ, Chen CJ, Wei CW, Lin CJ, Yiang GT, et al. Synergistic anti-tumor activity of isochaihulactone and paclitaxel on human lung cancer cells. J Cell Physiol 2012;227:213-22.